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THE MONONCHIDAE (ENOPLIDA: NEMATODA) OF NEW ZEALAND IV. THE GENUS *MYLONCHULUS* (COBB, 1916) PENNAK, 1953

BY

W. C. CLARK

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Two new species of *Mylonchulus* are described. *M. arenicolus* n. sp. is distinguished by its almost parallel-sided stoma, the arrangement of the subventral denticles and the tail shape. *M. ubis* n. sp. is marked off by the subterminal position of the caudal gland duct in conjunction with the regularly conoid tail and the arrangement of the subventral denticles.

The genus *Mylonchulus* appears to be cosmopolitan, having been recorded from most countries where free-living nematodes have been studied. Because Mulvey (pers. comm.) has a revision of the genus in the press, I am describing two new species without either reviewing the genus or commenting on the status of other species. A complete list of the Mononchidae recorded to date from the New Zealand region is appended. This is the last paper in this series (Clark 1960 a-d).

Mylonchulus arenicolus n. sp.

Figs. 1 A-D

Holotype ♀ L = 1.45 mm; a = 29; b = 3.59; c = 31.4; V ^{12}64.7 ^{13}
Paratype ♀ L = 1.48 mm; a = 31; b = 3.44; c = 29.7; V ^{14}62.7 ^{13}

Cuticle (head region) 1 μ thick, smooth; subcuticle 1 μ thick; cuticle much thicker on tail. No lateral fields. Lip region scarcely offset. Lips distinct, somewhat pointed; papillae six in inner ring and ten in outer ring. Amphid apertures appear as transverse slits in lateral view situated at the level of the dorsal tooth apex.

Stoma heavily sclerotized, almost parallel-sided anteriorly, pointed posteriorly, greatest width equal to three-fifths of stoma length, or half body width at this point. Dorsal stoma wall with a massive pointed dorsal tooth; anterior edge concave, sloping posteriorly from tooth apex; posterior edge convex, merging with stoma wall near middle of stoma. Tooth apex extending to middle of stoma and situated at 14—19 per cent of stoma length. Posterior dorsal stomal element rather triangular in optical section. Ventral wall similar to dorsal, but bearing 2 small subventral teeth and six rows of denticles arranged across the ventro-lateral stomal elements. Anterior and posterior ventral elements appear fused. Posterior quarter

[1]) on study leave from Entomology Division, D.S.I.R., Nelson, New Zealand.

of stoma with fine transverse striae. Posterior third of stoma embedded in slightly swollen anterior end of oesophagus. Oesophagus narrows a little to the nerve ring at one quarter of its length, then expands again, generally occupying two-thirds of the body width. Dorsal and subventral oesophageal gland ducts occur at 50, 63, and 94 per cent of oesophageal length. Oesophago-intestinal junction non-tuberculate, no transparent zone observed. Intestine usual for the family.

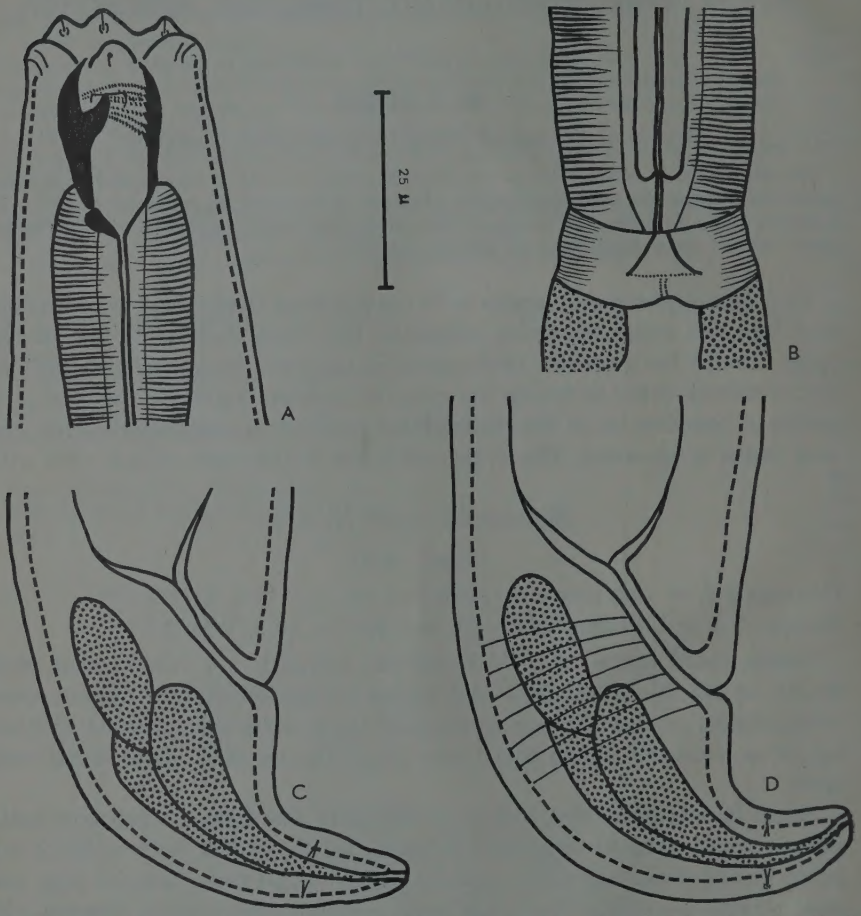

Fig. 1. *Mylonchulus arenicolus* n. sp. A. head region, B. oesophago-intestinal junction, C. tail of holotype, D. tail of paratype.

Ovaries paired, reflexed. Vulva a transverse slit at about two-thirds of body length. Vagina thick walled, extending across one-third of body width. Uteri outstretched with a well developed sphincter muscle at junction with each oviduct; spermatozoa occur abundantly on either side of the sphincter.

Tail short, conoid, ventrally arcuate, generally as in figure 1 C, but often as in fig. 1 D. Caudal glands well developed; duct terminal in position. Cuticular caudal pores as shown in figs. 1 C-D. Larvae similar to female.

Males unknown, but the abundance of sperm in the females (collected in February) indicates their probable existence.

Specific differentiation. Readily distinguished from the other New Zealand species by the terminal position of the caudal gland duct. *M. arenicolus* is distinguished from other species in the genus by its almost parallel-sided stoma, the arrangement of the sub-ventral denticles and the tail shape. It appears to be closest to *M. incurvus* (Cobb, 1917).

Type slides. Holotype and paratypes in Nematode collection, Entomology Division, D.S.I.R., N.Z.

Type locality. Kumara Beach, Westland, N.Z.M.S.I., S 50/51 741654 in unstabilized dune sand about the roots of *Desmoschoenus frondosus* Banks & Sol.

Mylonchulus ubis n. sp.

Figs. 2 A-E

Kumara Beach (Holotype) ♀ L = 1.24 mm; a = 29; b = 3.54; c = 23.1; V = ^{8} 60 ^{11}

(Paratypes) 2 ♀♀, L = 1.2-1.23 mm; a = 27-28; b = 3.36-3.49; c = 24.0-24.7; V $^{6-7}$ 59.5-60 ^{11}

Waitaanga Saddle 2 ♀♀ L = 1.07-1.09 mm; a = 30; b = 3.09-3.12; c = 21.5-23.1; V = 63

Kiripaka ♀ L = 1.10 mm; a = 27; b = 3.31; c = 22.0; V = ^{13} 63.5 ^{12}

Reefton ♀ L = 1.22 mm; a = 27; b = 3.51; c = 24.3; V = ^{7} 59 ^{11}

Ross ♀ L = 1.2 mm; a = 25; b = 3.49; c = 23; V = ^{19} 60.7 ^{12}

Wharekohe ♀ L = 1.31 mm; a = 32; b = 3.79; c = 26.2; V = ^{8} 54 ^{6}

Cuticle 1.3 μ thick, smooth, no lateral fields; subcuticle 1 μ thick. Lip region scarcely offset, lips well developed. "Inner lips" pointed, "outer lips" rounded. Papillae in two rings, six in the inner and ten in the outer ring. Amphid aperture a transverse slit situated slightly posterior to the dorsal tooth apex, one-fifth as wide as body at this level. Stoma goblet shaped, pointed posteriorly, half as wide as body in this region. Stoma dominated by the large anteriorly directed dorsal tooth whose apex reaches the centre of the stoma, and which occurs at 14-21 per cent of the length of the stoma. Anterior edge of tooth concave, posterior edge convex. Dorsal wall of stoma clearly divided into anterior and posterior elements, but subventral elements appear to be completely fused. Postero-dorsal element triangular on optical section, antero-dorsal element arcuate in outline. Subventral element straighter anteriorly than dorsal element. Subventral denticles in five to seven transverse rows. Posterior subventral denticles not conspicuously larger than the more anterior ones. Posterior part of stoma with fine transverse striae. Poste-

rior third of stoma embedded in locally expanded anterior end of oesophagus which narrows slightly to the nerve ring at a quarter of its length and then expands slightly in conformity with the body contour, generally occupying two-thirds of the body width. Dorsal and subventral oesophageal gland ducts discharge into the oesophageal lumen at 55, 68 and 93 per cent of the oesophageal length respectively.

Oesophago-intestinal junction non-tuberculate with a transparent zone intercalated between the posterior end of the oesophagus and the sphincter muscle. Intestine typical of the family.

Vulva at 54-63 per cent of body length. Ovaries paired, opposed, reflexed,

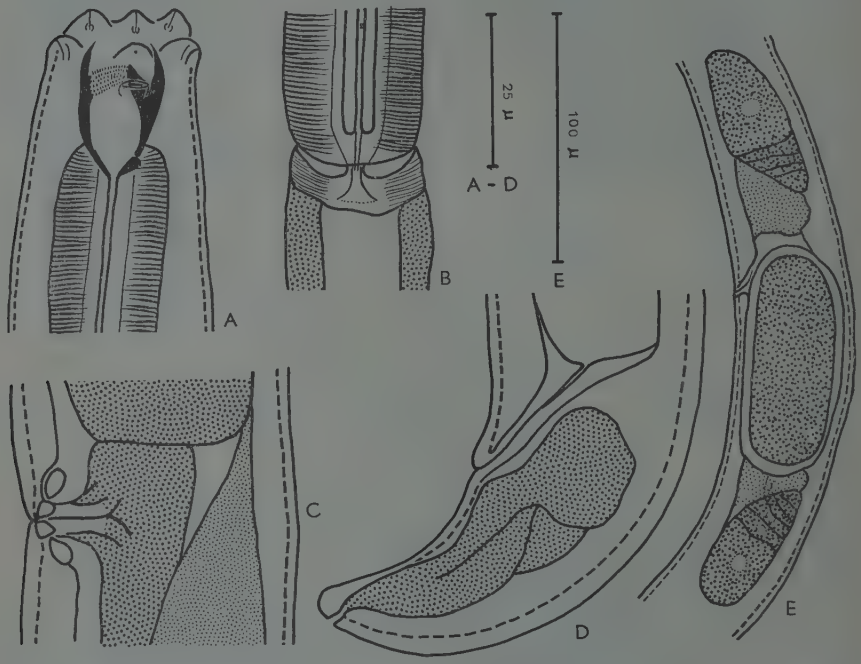

Fig. 2. *Mylonchulus ubis* n. sp. A. head region, B. oesophago-intestinal junction, C. vulva, D. female tail, E. reproductive organs.

occupying half body width. Oviducts and uteri short, with a sphincter muscle and glandular region at the oviduct-uterus junction. Only one mature ovum in the uterus at a time: there does not appear to be room for more in such a short reproductive tract. Egg length almost twice body width. Vagina with muscular walls extending across one-third of body width.

Prerectum absent, rectum straight, length equal to two-thirds anal-body-width.

Tail conoid, ventrally arcuate, tip rounded; caudal glands well developed, duct dorsally subterminal. Cuticular caudal pores apparently lacking.

Larvae resemble females.

Males unknown.

Specific differentiation. Readily distinguished from *M. arenicolus,* the only other known New Zealand species by the subterminal position of the caudal gland duct. The subterminal position of the caudal gland duct in conjunction with the regularly conoid tail, the regular arrangement of the subventral denticles and the absence of a pair of large, posterior subventral denticles, serve to distinguish this species from all others in the genus.

Type slides. Holotype and paratypes in nematode collection, Entomology Division, D.S.I.R., New Zealand. Paratype in slide collection, Nematology Department, Rothamsted Experimental Station, Harpenden, Herts., England, slide no. 147/4/1.

Type locality. Kumara Beach, Westland, N.Z.M.S.1. S 50 51 741656, in stabilized sand ridge with *Trifolium repens* L., *Hydrocotyle* sp., *Hypochoeris radicata* L., *Plantago sp.,* and *Festuca novaezealandiae* Cockayne. Soil: Mahinapua sands (imature podzolic loamy sands).

Other localities: Ross, Westland in *Trifolium repens* L. — *Anthoxanthum odoratum* L. — *Lolium perenne* L. pasture; Reefton in *Sphagnum* swamp with *Hypochoeris radicata* L.; Hongi's Track, in *Beilschmiedia Tawa* Benth. & Hook. f. — *Knightia excelsa* R. Br. forest litter; Waitaanga Saddle in liverworts and mosses in Tree-fern — *Brachyglottis repanda* Forst. — *Weinmannia racemosa* Linn. f. forest; Wharekohe, in 18 year-old *Paspalum dilatatum* Poir — *Lolium perenne* L. — *Trifolium repens* L. pasture; Kiripaka in *Beilschmiedia Taraire* Benth. & Hook. f. — *Vitex lucens* T. Kirk — *Dysoxylum spectabile* Hook. f. forest litter.

Remarks. All localities where this species has been found either are, or in historic time have been forested areas. This species has not been found in natural grasslands areas, but has been found in grasslands induced by man.

CHECKLIST OF NEW ZEALAND MONONCHIDAE

Mononchus composticola Clark, 1960
„ *mesadenus* Clark, 1960
„ *propapillatus* Clark, 1960
Prionchulus muscorum (Dujardin, 1845) Chitw. & Chitw., 1937
Mylonchulus ubis n. sp.
„ *arenicolus* n. sp.
Cobbonchus chauliodus Clark, 1960
Cobbonchus pounamua Clark, 1960
Miconchus rex (Cobb, 1904) Andrássy, 1958
Iotonchus basidontus Clark, 1960
„ *maragnus* Clark, 1960
„ *ophiocercus* Clark, 1960
„ *parazschokkei* (Allgen, 1929) Andrássy, 1958
„ *percivali* Clark, 1960

Allgen's (1929) record of *Iotonchus trichurus* (Cobb, 1917) Andrássy, 1958 from Campbell Island is considered doubtful.

ZUSAMMENFASSUNG

Die Mononchiden (Enoplida: Nematoda) Neu-Seelands
4. Die Gattung Mylonchulus *(Cobb, 1916) Pennak, 1953*

Zwei neue Arten der Gattung *Mylonchulus* werden beschrieben. *Mylonchulus arenicolus* n. sp. ist unterschieden durch den Besitz einer fast parallel-seitigen Mundhöhle, fünf Reihen subventraler Zähnchen, zwei kleiner subventraler Zähne und einem gebogenen fast konisch gefingerten Schwanz mit einer kaudalen Drüsengang. *M. ubis* n. sp. ist charakterisiert durch die Anwesenheit regulär angeordneter subventraler Zähnchen und dem Fehlen subventraler Zähne. Der Schwanz ist in der Regel konisch gebogen mit einer dorsalen, subterminalen Drüsengang.

REFERENCE

Allgen, C. (1929). Über einige freilebende Moos-Nematoden, *Nyt Mag. Naturvid.* **67**, 211-230.
Clark, W. C. (1960a). The oesophago-intestinal junction in the Mononchidae (Enoplida, Nematoda). *Nematologica* **5**, 178-183.
- - - (1960b). The Mononchidae (Enoplida: Nematoda) of New Zealand 1. The genera *Mononchus* Bastian, and *Prionchulus* Cobb. *Nematologica*, **5**, 199-214.
- - - (1960c). The Mononchidae (Enoplida: Nematoda) of New Zealand. 2. The genus *Iotonchus* (Cobb, 1916) Altherr, 1950. *Nematologica* **5**, 259-273.
——- (1960d). The Mononchidae (Enoplida: Nematoda) of New Zealand. 3. A review of the genus *Cobbonchus* Andrássy, 1958 with descriptions of new species, *Nematologica* **5**, 274-283.

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BROWNING OF CHRYSANTHEMUM LEAVES INFESTED WITH *APHELENCHOIDES RITZEMABOSI*

BY

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The rate of browning of infested chrysanthemum leaves increased with relative humidity and the number of eelworms. The eelworm is the primary cause of leaf discolouration although the brown sectors may contain a distinctive fungal population. Chlorogenic acid and isochlorogenic acid are the chief polyphenolic substrates for leaf browning. When cells are pierced by the eelworm stylet during feeding the polyphenols and the enzyme polyphenol oxidase probably meet resulting in oxidation and polymerisation to form the brown pigments.

The chemistry of leaf browning during the manufacture of tea and tobacco has been described by numerous authors. Plant pathogens may also cause discolouration of plant tissues and this subject has been studied by both mycologists and virologists. The nature of leaf discolouration caused by eelworms, however, has not been investigated. Hesling & Wallace (1960) have suggested that varietal susceptibility of chrysanthemums to *Aphelenchoides ritzemabosi* (Schwartz) may be associated with a hypersensitive browning reaction of the leaf. Before the problem of resistance or host-parasite relations can be studied, however, it is essential to know something of the factors which influence browning and the chemistry of the process.

MATERIALS AND METHODS

Chrysanthemum eelworms were obtained from dried infested chrysanthemum leaves. Leaves were infested by introducing a known number of eelworms on to the undersurface of the leaf by the method described previously (Wallace, 1960). Areas of browning on the leaf were measured by tracing on to paper and weighing the paper. The study of browning under sterile conditions was achieved by dipping leaves in a saturated calcium hypochlorite solution for 5 minutes followed by washing in sterile water. Eelworms were sterilised by immersion in a solution of 3.75 p.p.m. ethoxy-ethyl mercury chloride and 0.01% dioctyl sodium sulphosuccinate for 3 days (Crosse & Pitcher, 1952, 1953). The isolation and identification of fungi on the surface of infested leaves were made by Dr. F. T. Last using a technique already described by him (Last, 1955).

Polyphenols in the leaf were identified by paper chromatography. About 2 g of leaf were macerated in 80 per cent methyl alcohol in an Atomix 100 homogeniser for 1 minute. This was filtered and the filtrate concentrated in a rotary

evaporator under vacuum to give a final volume of 5 ml. After centrifugation the leaf extract was stored at 0°C. Descending chromatograms were run on No. 1 Whatman paper 46 × 57 cm in Shandon tanks. About 0.01 ml of the leaf extract was spotted on the paper. Two-way chromatograms were run with the organic phase of n-Butanol: acetic acid: water (4 : 1 : 5) and 6 per cent acetic acid as the solvents. After drying, the papers were examined under U.V. with and without ammonia fumes. Different sprays were also used to identify the spots, and finally the R_F values were measured. After provisional identification by these criteria, the spots were eluted from the paper by immersion in 80 per cent methyl alcohol for 24 hours and re-run one way against authentic specimens in four different solvents: 6% acetic acid, n-Butanol: acetic acid: water (4 : 1 : 5), glacial acetic acid: conc. HCl: water (10 : 1 : 3) and water. The leaves were also hydrolysed in 2N HCl for 1 hour, the solution filtered, concentrated by rotary evaporation,

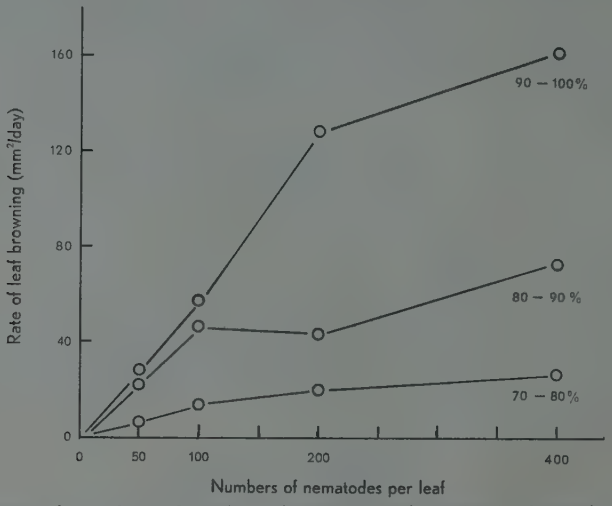

Fig. 1. Influence of humidity and numbers of eelworms on browning in chrysanthemum leaves.

centrifuged and finally spotted on to chromatography paper. Identification methods were similar to those for the unhydrolysed leaf extract. Spectrograms of the major spots on the papers were obtained using a Unicam U.V. spectrophotometer.

Isolation of polyphenol oxidase from leaves was made at 0° C. About 10 g of leaf was macerated in 70 ml of phosphate buffer (pH7). The leaf extract was centrifuged to remove plant debris and dialysed in distilled water for 24 hours. The enzyme solution was then removed from the dialysis bags, centrifuged and stored at 0° C.

RESULTS

Influence of humidity and numbers of eelworms on rate of browning

To measure quantitatively the influence of moisture on the rate of browning, leaves of variety Westfield Bronze were kept at three humidity levels and infested

with four levels of inoculum, 50, 100, 200 and 400 eelworms per leaf. Eight leaves were used in each treatment and at the end of 8 days the area of browning on each leaf was measured. The leaves were inserted by their petioles into damp peat in seed boxes and covered with polythene. Precise control of humidity was not achieved but by varying the degree of ventilation it was possible to obtain (1) a high humidity of 90 to 100 per cent , (2) an intermediate humidity of 80 to 90 per cent and (3) a low humidity of 70 to 80 per cent. Relative humidities

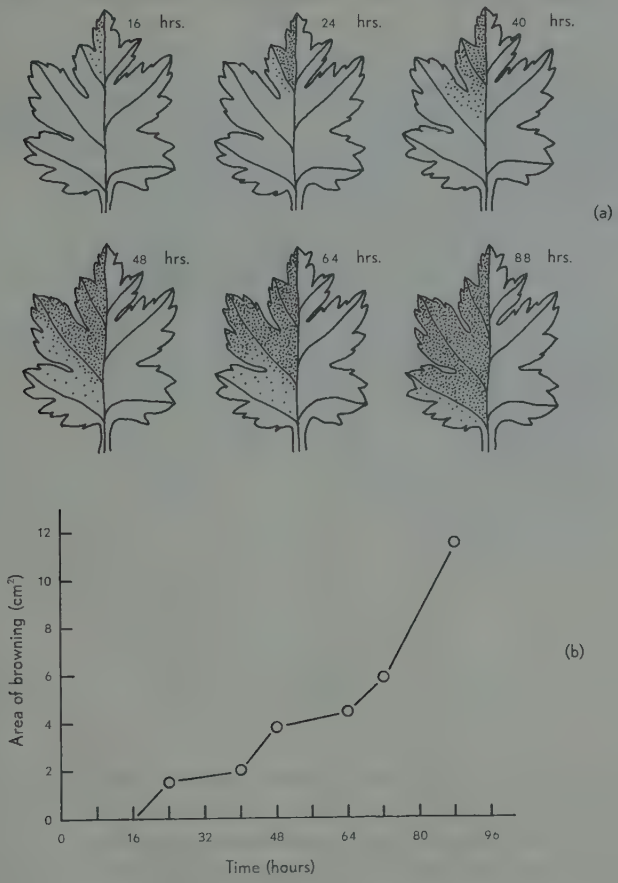

Fig. 2. (a) Pattern of browning in an eelworm infested chrysanthemum leaf at intervals over 88 hours. (b) Spread of browning in infested chrysanthemum leaves over 88 hours. Each point is the mean of ten replicates.

were measured with a hair hygrometer. The results are expressed in Fig. 1. The rate of browning increased with relative humidity and at each humidity with the number of nematodes. In experiments on leaf browning it is, therefore, necessary

to control humidity and inoculum level. The marked increase in the rate of spread of symptoms in leaves and up the plant during early autumn is probably due to the higher humidity and occurrence of persistent mists at that time of the year.

Patterns of infested leaves and rate of browning

Ten leaves of variety Amy Shoesmith were each infested at the tip on one side of the main vein with about 200 eelworms. The areas of leaf which were completely brown were plotted at intervals over 88 hours and the results are shown diagramatically in Fig. 2a and graphically in Fig. 2b. The first symptoms appear in about 8 hours and browning spreads from the tip to the petiole on one side of the main vein. The graph of area of brown leaf with time is not linear but consists of a series of small curves. Browning is not a continuous process because the lateral veins act as barriers to eelworm migration within the leaf. Thus, the sector at the tip of the leaf turns completely brown in 24 hours, the

Fig. 3. Leaf browning in three varieties of chrysanthemum leaves. S — leaf surface and eelworms sterilized, N — leaves and eelworms unsterilized.

next sector shows faint symptoms by this time but is only completely brown after 48 hours. The next sector takes another 40 hours before complete browning occurs. This intermittent spread of symptoms gives the familiar picture of leaf colouration diagnostic of chrysanthemum eelworm infestation. The fact that nearly half the leaf is completely brown at the end of 4 days means that the progeny of the original females introduced on to the leaf are isolated within those sectors and are, therefore, liable to desiccation at low humidities. The rate of browning is therefore important in the survival of larvae in the leaf.

Fungi associated with the browning leaf

It has been assumed that eelworms are the sole cause of browning in infested chrysanthemum leaves. It is possible, however, that other organisms, fungi or

H. R. WALLACE: *Browning of Chrysanthemum leaves infested with* Aphelenchoides ritzemabosi.

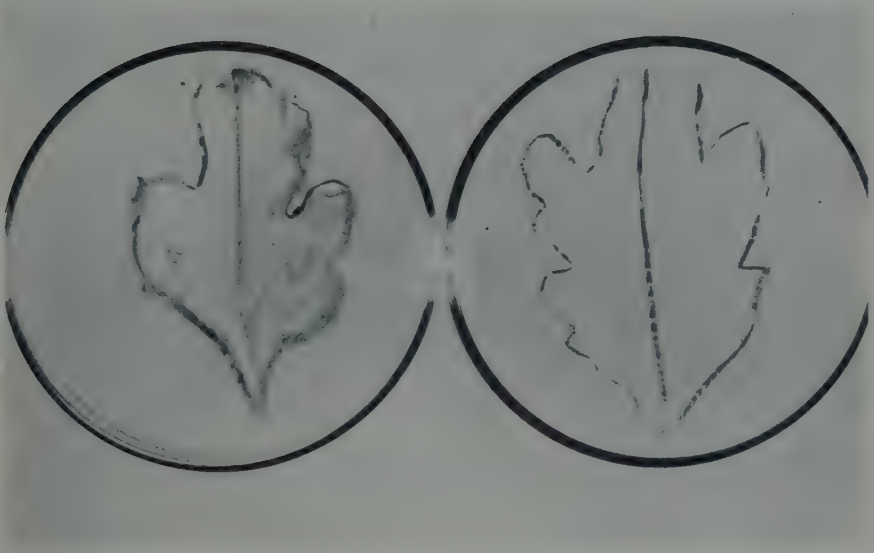

Fig. 4. The saprophytic fungus *Sporobolomyces roseus* associated with browned sectors of an eelworm infested chrysanthemum leaf. *Left* — leaf infested on right hand side of main vein. *Right* — uninfected leaf. The outline of the leaves has been drawn in with a pencil.

bacteria, may also contribute to the symptoms. Eelworms were removed from a water suspension and the remaining solution introduced on to the undersurface of twenty leaves by the polythene strip method. No symptoms appeared. In twenty similar leaves with eelworms in the inoculum, clear symptoms appeared in 24 hours in every leaf.

Five leaves of each of the varieties Delightful, Orange Peach Blossom and Amy Shoesmith were surface-sterilised and suspended in a glass tank with their petioles in water. Each leaf was then infested with 200 sterile eelworms. A similar experiment was set up with non-sterile leaves which were inserted into peat by their petioles and covered with polythene. These leaves were infested with non-sterile nematodes. The areas of browning on the leaves were measured after 11 days. The results (Fig. 3) indicate no difference between treatments. There

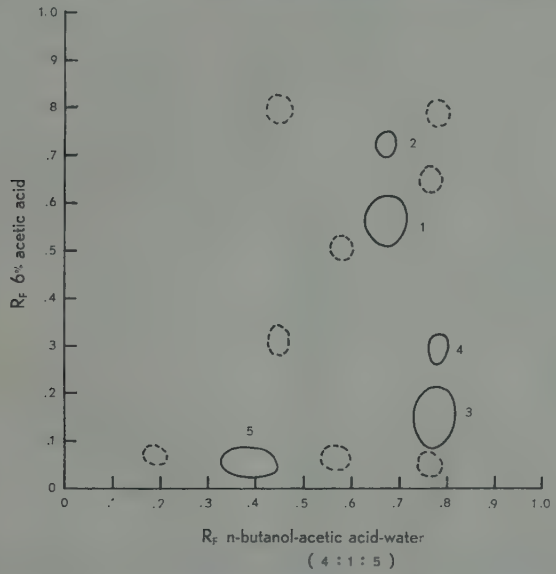

Fig. 5. Chromatogram of chrysanthemum leaf extract showing major spots 1-5 and faint spots (dotted outlines).

was a marked relationship between the brown sectors and the presence of the saprophytic fungus *Sporobolomyces roseus* Kluyver & v. Niel (Fig. 4). This technique does not reveal all the fungi on the leaf surface and none within the leaf, however, it seems likely that browned parts of leaves have a different micro-flora and fauna from the green parts. These micro-organisms may accentuate symptoms but there is no evidence that they are capable of causing leaf discolouration in the absence of the eelworm. To test this hypothesis twenty leaves of variety Orange Peach Blossom were treated in the following way: five leaves were each infested with 200 eelworms in a concentrated spore suspension of *Sporobolomyses roseus*,

five leaves received 200 eelworms each without fungus, five leaves received spore suspension but no eelworms and five leaves were untreated. The mean area of browning for each treatment was determined after 4 days. In the absence of eelworms, no symptoms appeared. Infestations with eelworms plus fungus gave a brown area of 11.7 sq. cm, nematodes without fungus 11.5 sq. cm. These two values were not significantly different. This result supports the hypothesis that chrysanthemum eelworm is probably responsible for most of the browning symptoms. The symptomatology of leaves grown under completely sterile conditions, however, is needed to assess the true role of the eelworm.

TABLE I

R_F values of major polyphenols in different solvents

Spot No.	1	2	3	4	5
6% acetic acid	.57	.72	.15	.29	.05
n-butanol: acetic acid: water (4 : 1 : 5)	.67	.67	.78	.78	.39
water	.58	.78	.09	.20	.04
glacial acetic acid: conc. HCl: water (10 : 1 : 3)	.81	.81	.60	.60	.40
	Colour reactions to different sprays				
NH_3 fumes	fY	—	fY	—	Y
U.V.	B	fB	B	B	Br
U.V. plus NH_3 fumes	bBG	bBG	bBG	bBG	bY
diazotised p-nitroaniline	OR	—	OR	—	fO
1% ethanolic $FeCl_3$	GGr	—	GGr	—	G
1% ethanolic $AlCl_3$	Y	Y	Y	Y	BrY
Hoepfner reagent	P	P	P	P	BrO
$AgNO_3$ plus NH_3	Br	fBr	Br	fBr	Br
Vanillin in ethanol	—	—	—	—	Y

f — faint; b — bright; Y — yellow; B — blue; Br — brown; G — green; O — orange; R — red; Gr — grey; P — pink.

The chemical nature of browning

Browning in leaves of several plant species has been shown to be caused by an enzymic oxidation of polyphenols to quinones and subsequent polymerisation to brown substances. To study browning in eelworm infested chrysanthemum leaves it was assumed that similar chemical processes occurred. Two way chro-

TABLE II

R_F values of major polyphenols from hydrolysed leaves in different solvents

Spot No.	1	Caffeic acid (authentic)	2	Luteolin (authentic)
6% acetic acid	.32	.32	.02	.02
glacial acetic acid: conc. HCl: water (10 : 1 : 3)	.66	.66	.33	.34
n-butanol: acetic acid: water (4 : 1 : 5)	.82	.82	—	—

matograms of leaf extracts of ten varieties of chrysanthemum showed the presence of five major spots and several faint spots under U.V. with ammonia fumes. No qualitative differences were evident between varieties. A diagram of the spots is shown in Fig. 5. The colour reactions to different sprays, and the R_F values of the five major spots in four solvents is shown in Table I. Spots 1 and 2 were identified as the cis- and trans-forms of chlorogenic acid, spots 3 and

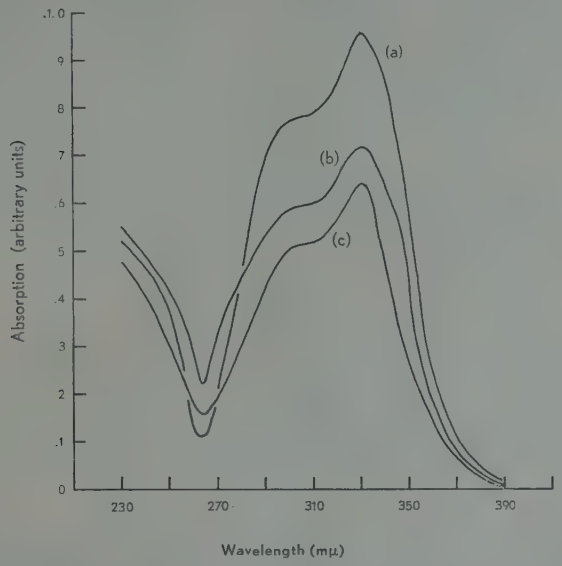

Fig. 6. U.V. absorption spectra of (a) chlorogenic acid and (b) isochlorogenic acid eluted from chromatograms and of an authentic specimen of chlorogenic acid (c).

4 as the cis- and trans-forms of isochlorogenic acid and spot 5 as a glycoside of luteolin. Spots 1 and 2 eluted from the chromatogram and run against authentic (commercial) chlorogenic acid gave similar R_F values in the four solvents. Spots 3 and 4 had similar R_F values to isochlorogenic acid. No authentic specimen of the glycoside of luteolin was available. Hydrolysis of the leaf extracts yielded two major spots on a two way chromatogram, which were identified as caffeic acid and luteolin. The spots were eluted and run against authentic specimens and agreement in R_F values was close for both substances (Table II). Caffeic acid is the hydrolysis product of chlorogenic acid and isochlorogenic acid and luteolin of the luteolin glycoside, thus supporting the conclusions on the identities of spots 1 to 5. Spectrograms of spots 1 and 3 agreed closely with that of authentic chlorogenic acid (Fig. 6). Evidence from R_F values, colouration by various sprays, co-chromatography with authentic specimens, spectrography and identification of hydrolysates confirm the identity of the major spots.

To determine the polyphenols responsible for browning, one way chromato·

grams were run in 6 per cent acetic acid, dried and then sprayed with polyphenol oxidase solution prepared from chrysanthemum leaves. Browning of the chlorogenic acid and isochlorogenic acid spots was clearly visible but there was no such effect on the luteolin glycoside or any other spot. Thus, chlorogenic acid and isochlorogenic acid are probably the chief substrates for browning in the chrysanthemum leaf. Further evidence for this was obtained by bubbling air through a concentrated leaf extract in phosphate buffer (pH7) at room temperature. The solution was sampled at intervals and spotted on chromatography paper

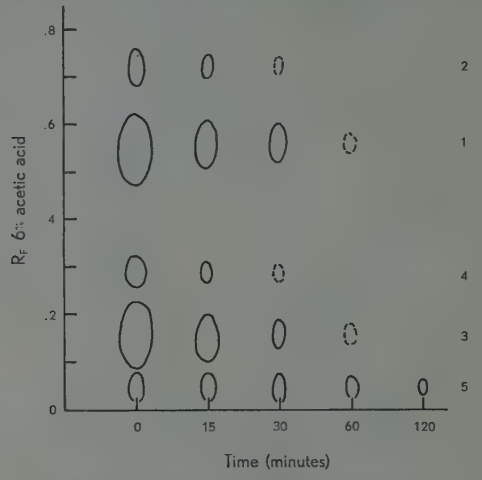

Fig. 7. Diagram showing changes in size and intensity of major spots in one-way chromatograms of chrysanthemum leaf extract in aerated phosphate buffer at various time intervals.

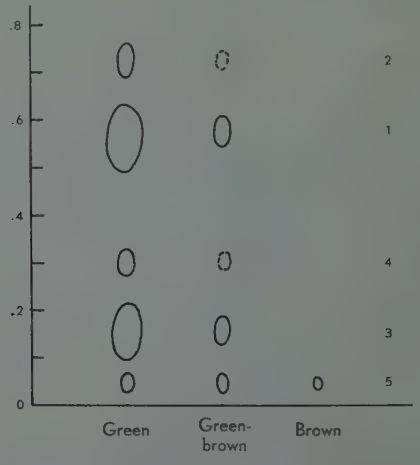

Fig. 8. Diagram showing size and intensity of major spots in one-way chromatogram of extracts from green, brown-green and brown parts of infested chrysanthemum leaves.

which was subsequently run in 6 per cent acetic acid (Fig. 7). At zero time the five major spots were clearly visible. After 15 minutes there was little change but by 30 minutes the size and intensity of the chlorogenic acid and isochlorogenic acid spots were reduced but there was no reduction in the luteolin glycoside spot. After 1 hour the chlorogenic and isochlorogenic acid spots were just visible but had completely disappeared after 2 hours. The luteolin glycoside spot showed slight reduction over this time. The disappearance of the spots coincided with a browning of the aerated leaf extract.

Chromatograms of extracts from infested leaves showed the presence of the five major spots in the green tissues. A marked reduction in the spots was evident in the brown-green parts of the leaf and the dark brown portions showed only a faint luteolin glycoside spot (Fig. 8).

These experiments show that browning in the leaf is associated with a reduction in the amounts of chlorogenic acid and isochlorogenic acid, which are probably the chief substrates for enzymic oxidation by polyphenol oxidase.

DISCUSSION

Experiments indicate that chrysanthemum eelworm is a true pathogen and probably the primary cause of browning in the infested leaf. However, the question of how the eelworm does this is still unanswered. It is known that the eelworm feeds by inserting the mouthspear into cells and it is likely that there is mechanical damage during the process. Polyphenols and the enzyme polyphenol oxidase are separated in the healthy leaf and so it is possible that during feeding polyphenols and enzyme meet resulting in oxidation and polymerisation to give the brown pigments. Mechanical damage to leaf cells was made with a fine needle resulting in browning of the damaged cells. There was no spread of the browning. It is possible that the eelworms secrete polyphenol oxidase while feeding but attempts to demonstrate this enzyme in the chrysanthemum eelworm have so far failed. There is no evidence, therefore, which contradicts the hypothesis that browning is caused by mechanical damage to the cell during feeding.

I am indebted to Dr. Bate-Smith for an authentic specimen of luteolin and to Dr. Williams for a mixture of chlorogenic acid and isochlorogenic acid.

ZUSAMMENFASSUNG

Braunfärbung von Chrysanthemumblätter durch Aphelenchoides ritzemabosi

Die Braunfärbung befallener Chrysanthemumblätter steigert sich mit relativer Feuchtigkeit und Zahl der Älchen. Die Bräunung ist kein kontinuierlicher Vorgang, weil die seitlichen Blattadern als Begrenzung für die Wanderung der Älchen wirken. Sterilisieren der Blattoberfläche und der Älchen vermindert die Stärke der Braunfärbung nicht Hieraus wird geschlossen, dass das Älchen die primäre Ursache der Blattverfärbung ist. Der saprophytische Pilz *Sporoholomyces roseus* ist eng mit den braun gefärbten Blatteilen verbunden, trägt aber zur Verfärbung nicht bei. Chlorogensäure, Isochlorogensäure und ein Glykosid von Luteolin sind die wichtigeren Polyphenol-Bestandteile der Chrysanthemumblätter. Chlorogensäure und Isochlorogensäure sind die Hauptsubstrate für Blattbräunung. Es ist anzunehmen, dass, wenn die Zellen von dem Mundstachel der Älchen angebohrt werden, die Polyphenole und die enzymatische Polyphenoloxydase zusammentreffen und durch Oxydation und Polymerisation die braunen Flecke bilden.

REFERENCES

CROSSE, J. E. & PITCHER, R. S. (1952). Studies in the relationship of eelworms and bacteria to certain plant diseases. I. The etiology of strawberry cauliflower disease. *Ann. appl. Biol.* **39**, 475-484.

—— & —— (1953). A preliminary note on methods for obtaining bacteria-free eelworms. *Rep. E. Malling Res. Sta. for 1957*, 144-147.

HESLING, J. J. & WALLACE, H. R. (1960). Susceptibility of varieties of chrysanthemum to infestation by *Aphelenchoides ritzema-bosi* (Schwartz), *Nematologica* **5**, 297-302.

LAST, F. T. (1955). Seasonal incidence of *Sporobolomyces* on cereal leaves. *Trans. Brit. mycol. Soc.* **38**, 221-239.

WALLACE, H. R. (1959). Movement of eelworms. V. Observations on *Aphelenchoides ritzema-bosi* (Schwartz, 1912) Steiner, 1932 on florists' chrysanthemums. *Ann. appl. Biol.* **47**, 350-360.

—— (1960). Observations on the behaviour of *Aphelenchoides ritzema-bosi* (Schwartz) in chrysanthemum leaves. *Nematologica* **5**, 315-321.

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Nematologica 6 (1961): 17-24. Leiden, E. J. Brill

ÜBER DIE SPEKTROPHOTOMETRISCHE BESTIMMUNG VON SCHLÜPFSTOFFEN FÜR *HETERODERA ROSTOCHIENSIS* WOLL. NACH ELLENBY. 4. MITTEILUNG ÜBER *HETERODERA*-ARTEN *)

VON

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Die von Ellenby beschriebenen Methoden zur spektrophotometrischen Bestimmung des „hatching factor" mit Hilfe alkalischer Pikrinsäure - bzw. 3,5-Dinitro-benzoesäure-Lösung wurden zur Untersuchung von Diffusaten mehrerer Solanaceen angewendet. Hierbei ergab sich, daß nicht nur schlüpfaktivierende Diffusate (Kartoffel-Wurzeldiffusat), sondern auch den Schlüpfvorgang nicht beeinflussende Wurzeldiffusate von *Solanum capsicastrum* sowie *Fabiana imbricata* und sogar schlüpfhemmende Diffusate von Tabakwurzeln und -blättern gleichartige Absorptionsmaxima zeigen. Die Anwendbarkeit der spektrophotometrischen Methoden sowie die mit diesen erzielten Ergebnisse werden diskutiert.

Die gebräuchlichen biologischen Testmethoden zum Nachweis und zur Bestimmung von Schlüpf- und Antischlüpfstoffen für den Kartoffelnematoden, *Heterodera rostochiensis* Woll., in Wurzelablaufwässern und Diffusaten bzw. Extrakten pflanzlicher Organe sowie von entsprechenden Wirkstoffkonzentraten und -präparaten (z.B. Eclepsäure) sind sehr zeitraubend und mit großem Arbeitsaufwand verbunden (Fenwick, 1949, 1952; Fenwick & Widdowson, 1958; Widdowson & Wiltshire, 1958; Sembdner & Schreiber, 1960). Darüber hinaus kann auch die bei allen biologischen Testen in Erscheinung tretende Abhängigkeit des Versuchsobjektes von zahlreichen biotischen und abiotischen Faktoren erschwerend wirken.

Auch die Beobachtung von Ellenby & Gilbert (1957; vgl. auch Ellenby 1957), daß den Schlüpfstoffpräparaten eine herzaktive Wirkung zukommt, womit unter gewissen Voraussetzungen eine Testung am isolierten Froschherzen ermöglicht wird, brachte in dieser Hinsicht keine wesentlichen Vorteile. Im Hinblick auf diese herzaktive Wirksamkeit der Eclepsäure und ihre voraussichtliche Lacton-Struktur erschien es Ellenby aussichtsreich, die für Herzglykoside bekannten chemischen Bestimmungsmethoden auch bei Wurzeldiffusaten und Schlüpfstoffpräparaten anzuwenden.

Tatsächlich ließen sich mit der Pikrinsäuremethode nach Baljet sowie mit dem 3,5-Dinitro-benzoesäure-Verfahren nach Kedde Ergebnisse erzielen, die wesentliche Erleichterungen bei der Bearbeitung biologischer und chemischer Fragen zur Schlüpfaktivierung des Kartoffelnematoden versprachen (Ellenby, 1958 a und c). Im Rahmen unserer Arbeiten auf diesem Gebiet überprüften wir die Spezifität

*) 3. Mitteilung: K. Schreiber & G. Sembdner, Planta Medica **8**, 107-113 (1960).

dieser Methoden sowie ihre Anwendbarkeit bei Untersuchungen über die unterschiedliche Wirkung von Diffusaten einiger Solanaceen auf den Schlüpfvorgang.

MATERIAL UND METHODIK

Zur Herstellung von Wurzeldiffusaten (WD) wurden die im Gewächshaus angezogenen Pflanzen ausgetopft und mit den sauber gewaschenen Wurzeln 24

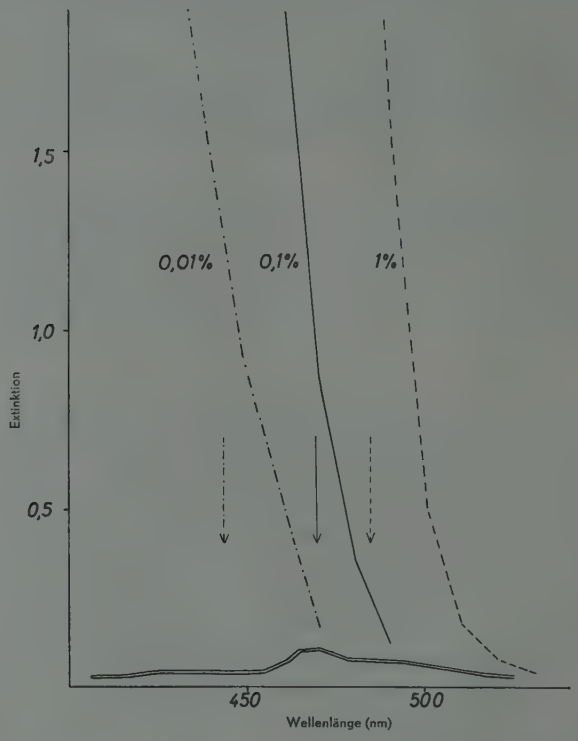

Abb. 1. Eigenabsorptionsspektren des Pikrinsäure-Reagenzes bei Verwendung von 95 ml 1,0-proz. (- - - -), 0,1-proz. (——) bzw. 0,01 proz. Pikrinsäure-Lösung (—·—·—) und 5 ml 10-proz. Natronlauge gegen Wasser als Kompensationsflüssigkeit.
(═══) Absorptionsspektrum von Kartoffel-WD mit Pikrinsäure-Reagenz (0,1-proz. Pikrinsäure) gegen ein Wasser-Reagenzgemisch als Kompensationslösung.
Die senkrechten Pfeile kennzeichnen die Lage der Absorptionsmaxima von Kartoffel-WD mit Reagenz unterschiedlicher Pikrinsäure-Konzentration.
Die Extinktionen wurden unter Verwendung von 2 cm-Küvetten ermittelt.

Stdn. lang in 100 ml Aqua dest. je Pflanze eingestellt. Untersucht wurden das schlüpfaktivierende Diffusat von *Solanum tuberosum* "Aquila" (Kartoffel-WD), das hemmstoffhaltige Diffusat von *Nicotiana tabacum* "Virgin Gold A" (Tabak-WD) sowie die in ihrer Wirkung auf den Schlüpfvorgang weitgehend indiffe-

renten Wurzeldiffusate von *Solanum capsicastrum* Link und von *Fabiana imbricata* Ruiz. et Pav. Das stark schlüpfhemmende Blattdiffusat von *Nicotiana tabacum* "Virgin Gold A" (Tabak-BD) wurde durch 24stündiges Einlegen von 135 g frisch geernteten Blättern in 500 ml Aqua dest. gewonnen. Außerdem untersuchten wir 0,025-proz. und 0,05-proz. wäßrige Lösungen von Gibberellin (C. Roth,

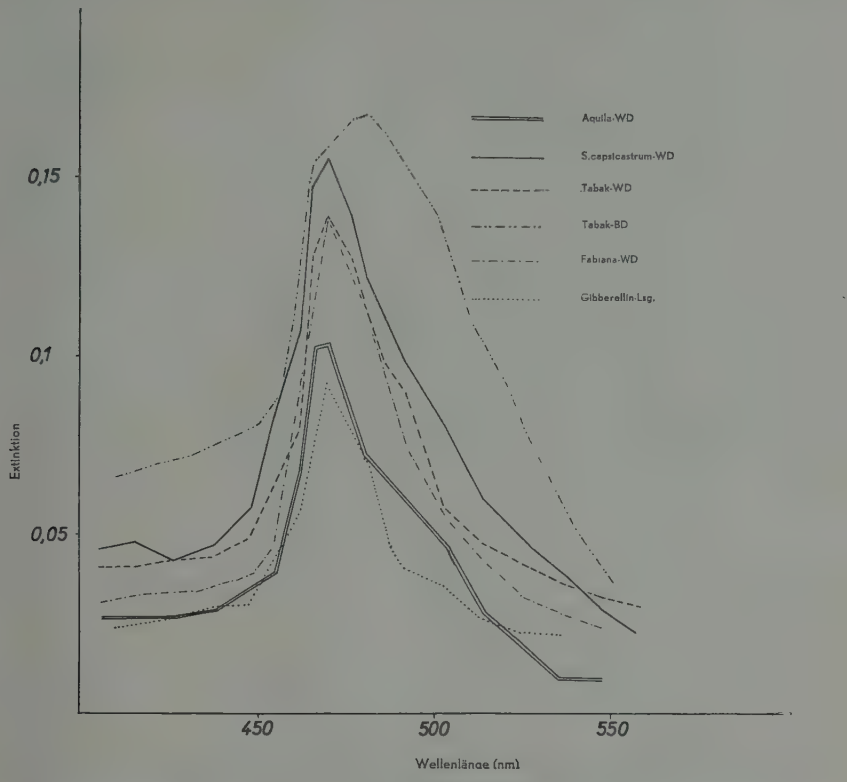

Abb. 2. Absorptionsspektren von Diffusaten bzw. 0,05-proz. Gibberellin-Lösung (8 ml) mit jeweils 4 ml Pikrinsäure-Reagenz (0,1-proz. Pikrat-Lösung).
Die Extinktionen wurden unter Verwendung von 2 cm-Küvetten und einer Wasser-Reagenzmischung als Kompensationslösung ermittelt.

Karlsruhe). Nach der papierchromatographischen und IR-spektroskopischen Untersuchung ist der Hauptbestandteil dieses Präparates Gibberellinsäure.

Für die spektrophotometrische Untersuchung der genannten Diffusate (bzw. der Gibberellin-Lösung) wurden unter weitgehender Berücksichtigung der Angaben von Ellenby (1958b) die von Rowson (1952, 1955) sowie Rowson & Dyer (1952) ausführlicher beschriebenen Digitoxin-Bestimmungsmethoden mit Hilfe alkalischer Pikrinsäure-Lösung bzw. alkalischer 3,5-Dinitro-benzoesäure-Lösung

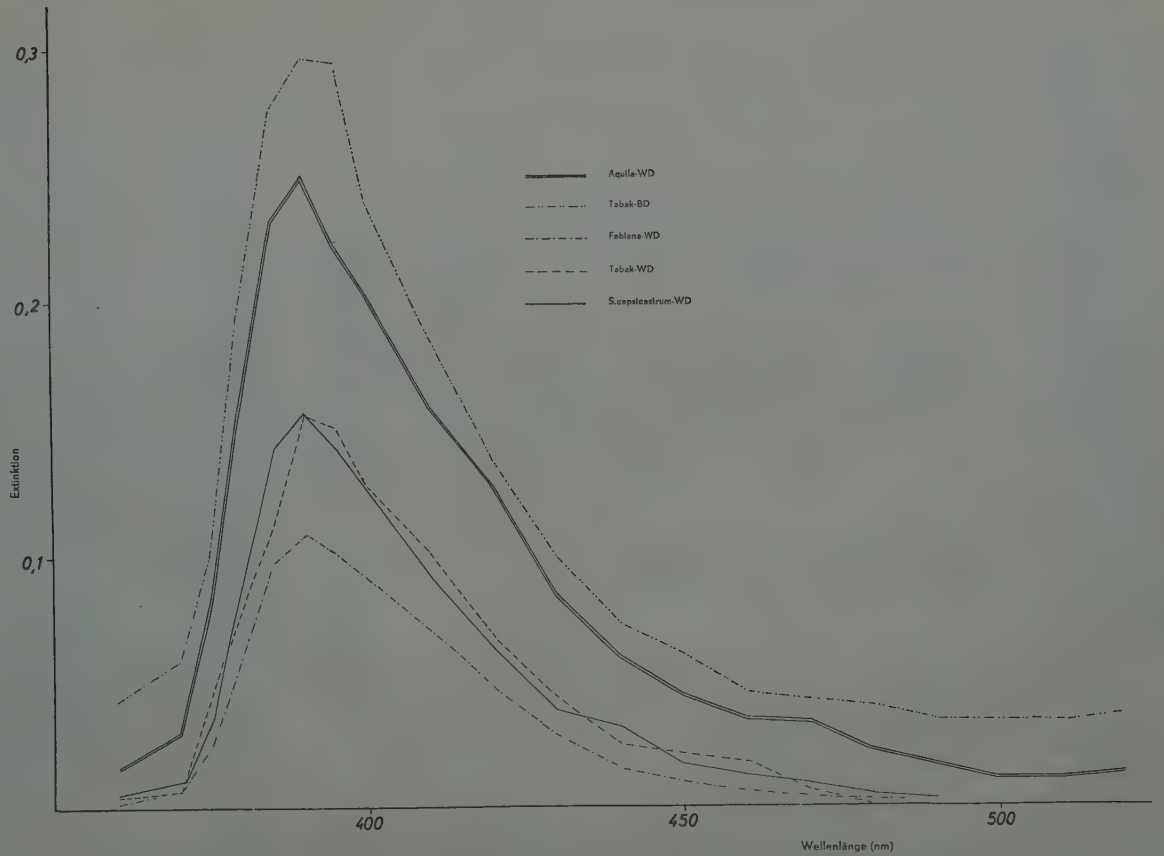

Abb. 3. Absorptionsspektren von Diffusaten (4 ml) mit Dinitro-benzoesäure-Reagenz (ml 2-proz. Äthanol. 3,5-Dinitro-benzoesäure + 2 ml 2n Natronlauge).
Die Extinktionen wurden nach 1 Stde. unter Verwendung von 1 cm-Küvetten und einer Wasser-Reagenzmischung als Kompensationslösung ermittelt.

angewendet. Die Extinktionen bei den jeweiligen Absorptionsmaxima wurden mit dem Universal-Spektrophotometer UVS1 des VEB C. Zeiss, Jena, unter Verwendung des Flintglasprismas, einer Glühlampe als Strahlenquelle sowie der Photozelle MQVS gemessen.

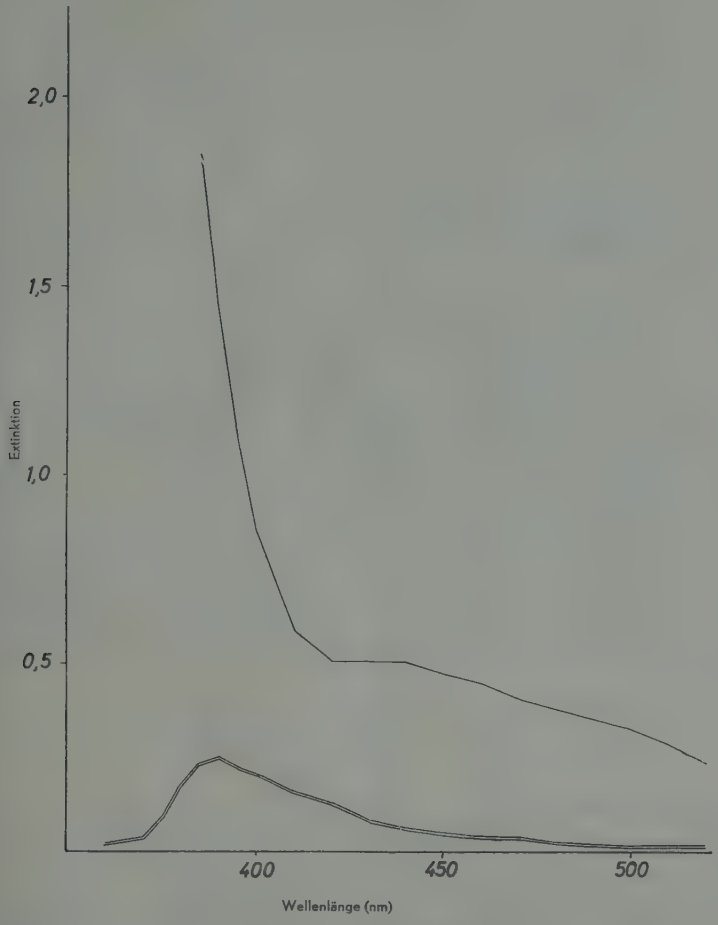

Abb. 4. Eigenabsorptionsspektrum des Dinitro-benzoesäure-Reagenzes (———) gegen Wasser als Kompensationsflüssigkeit.
(———) Absorptionsspektrum von Kartoffel-WD mit Dinitro-benzoesäure-Reagenz gegen ein Wasser-Reagenzgemisch als Kompensationslösung
Die Extinktionen wurden unter Verwendung von 1 cm-Küvetten ermittelt.

ERGEBNISSE

Pikrinsäure-Methode: Bei Verwendung von Kartoffel-WD ließen sich die Angaben von Ellenby hinsichtlich der Lage des Absorptionsmaximums (480 nm)

gut reproduzieren; auch die Größenordnung der Extinktion konnte im wesentlichen bestätigt werden. Jedoch beobachteten wir bei wiederholten Messungen mit einheitlichem Kartoffel-WD erhebliche Extinktionsschwankungen. Diese haben ihre Ursache in einem starken Anstieg der Eigenextinktion des Reagenzes von 500 nm nach kürzeren Wellenlängen hin, wodurch die Genauigkeit der Messung erheblich beeinträchtigt wird. Dieser störende Einfluß der starken Eigenextinktion konnte auch durch Herabsetzen der Pikrinsäure-Konzentration nicht ausgeschaltet werden, da hiermit eine Verschiebung des Absorptionsmaximums des WD/Reagenzgemisches verbunden ist (470 nm bei 0,1 %, 444 nm bei 0,1 % Pikrinsäure; vgl. Abb. 1).

Bei der Überprüfung der Diffusate weiterer Solanaceen (Abb. 2) stellten wir fest, daß entgegen unserer Erwartung auch die den Schlüpfvorgang hemmenden Diffusate von Tabakwurzeln und -blättern sowie die weitgehend unwirksamen Wurzeldiffusate von *Solanum capsicastrum* und *Fabiana imbricata* gleiche Absorptionsmaxima zeigen. Ein gleichartiges Absorptionsspektrum ermittelten wir auch mit 0,05-proz. Gibberellin-Lösung (Abb. 2).

3,5 Dinitro-benzoesäure-Methode: Die mit den gleichen Diffusaten und dem 3,5-Dinitro-benzoesäure-Reagenz aufgenommenen Absorptionsspektren werden in Abb. 3 dargestellt. Auch hier ließen sich die Angaben von Ellenby bezüglich Kartoffel-WD im wesentlichen bestätigen. In ähnlicher Weise wie mit der Pikrinsäure-Methode konnten wir auch bei den weiteren Diffusaten gleiche, wenn auch in ihrer Höhe unterschiedliche Absorptionsmaxima bei 385 nm feststellen. Dagegen zeigte Gibberellin-Lösung in den angewendeten Konzentrationen im Gegensatz zur Pikrinsäure-Methode kein Maximum bei dieser Wellenlänge. Auch bei der Dinitro-benzoesäure-Methode liegen die in Abb. 3 wiedergegebenen Absorptionsmaxima im Bereich der starken Eigenabsorption des Reagenzes (s. Abb. 4).

DISKUSSION

Wie unsere Untersuchungen gezeigt haben, sind die von Ellenby (1958b) beschriebenen und von uns bestätigten Maxima der Absorptionsspektren von Kartoffel-WD mit Pikrinsäure- bzw. Dinitro-benzoesäure-Reagenz nicht für die in diesem Diffusat vorkommenden Schlüpfstoffe (Eclepsäure) spezifisch, da auch nicht schlüpfaktivierende und sogar antischlüpfstoffhaltige Diffusate anderer Pflanzen ein gleichartiges Verhalten zeigen. Ähnliches wurde bereits von Ellenby (1958a) bei der spektrophotometrischen Untersuchung von *Digitalis*-Wurzeldiffusat (Dinitro-benzoesäure-Methode) beobachtet, ohne daß jedoch eine Deutung bzw. eine Verallgemeinerung dieses Befundes erfolgte. In mehrfach wiederholten Schlüpfversuchen erwiesen sich Wurzeldiffusate von *Digitalis purpurea* L. wie auch von *Solanum capsicastrum* und *Fabiana imbricata* als weitgehend indifferent, besaßen also weder Schlüpf- noch Antischlüpfstoffe in nachweisbaren Mengen.

Die Frage nach der Ursache für diese Erscheinungen, also die Frage nach der

Natur der Stoffe bzw. Stoffgruppen, die für die Ausbildung der Absorptionsmaxima verantwortlich sind, kann durch diese Untersuchungen nicht beantwortet werden. Offensichtlich dürfte es sich hier um Vertreter einer weit verbreiteten Gruppe von Pflanzenstoffen handeln, oder um unterschiedliche Verbindungen, die sich durch das Vorhandensein einer bestimmten, das spezifische Absorptionsspektrum bedingenden funktionellen Gruppe auszeichnen. Inwieweit durch diese Befunde unsere bereits früher geäußerte Vermutung gestützt wird, daß Schlüpf- und Antischlüpfstoffe eine ähnliche chemische Konstitution besitzen und möglicherweise den Gibberellinen nahestehen, muß vorerst noch offen bleiben. Bemerkenswert ist in diesem Zusammenhang, daß bei dem von uns geprüften Gibberellin mit der Pikrinsäure-Methode, jedoch nicht mit dem Dinitro-benzoesäure-Reagenz ein gleiches Absorptionsmaximum wie bei den Diffusaten auftritt. Damit wird gleichzeitig die Frage aufgeworfen, ob mit beiden Methoden die gleichen Stoffe erfaßt werden.

Durch die dargelegte Unspezifität der Farbreaktionen wird die Anwendungsmöglichkeit dieser Methoden zur Bestimmung des Schlüpfstoffgehaltes von Diffusaten erheblich eingeschränkt, wenn auch Untersuchungen mit begrenzter Fragestellung an Einzelobjekten, beispielsweise über die Menge der von Wurzeln ausgeschiedenen Stoffe schlechthin, nicht ausgeschlossen sind. Allerdings scheint die Methodik für die Erfassung quantitativer Unterschiede wenig geeignet, was insbesondere durch die starke Eigenabsorption beider Reagenzien im spektralen Untersuchungsbereich begründet sein dürfte. Diese Einschränkung gilt jedoch nicht für die Verwendung der Dinitro-benzoesäure-Methode zur Bestimmung herzaktiver Glykoside, wie beispielsweise Digitoxin, da wegen der anderen Lage des für die spektrophotometrische Bestimmung herangezogenen Haupt-Absorptionsmaximums bei 535 nm die genannten Schwierigkeiten nicht in Erscheinung treten.

Für technische Mitarbeit bei den spektrophotometrischen Untersuchungen danken wir Fräulein H. Detering.

SUMMARY

Comments on Ellenby's spectrophotometric estimation of the hatching factor of the potato root eelworm, Heterodera rostochiensis *Woll.*

Ellenby has described spectrophotometric methods for the assay of the hatching factor. These estimations with the alkaline picric acid reagent (absorption maximum at 480 nm) and with the 3.5-dinitro-benzoic acid reagent respectively (absorption maximum at 385 nm) have been extended to diffusates of various Solanaceae. Similar absorption spectra were obtained not only with active diffusates (potato-root diffusate) but also with non-active root diffusates of *Solanum capsicastrum* and *Fabiana imbricata* as well as with diffusates of tobacco roots and leaves, which inhibit hatching. Gibberellic acid too reacted with the picric acid reagent in a similar manner.

These results show that the above mentioned absorption maxima are not specific for the hatching factor. Therefore the application of this method for estimating the content of eclepic acid in diffusates seems very limited. Also, the methods appear unsuitable for quantitative determinations because of the strong self-absorption of both reagents in the spectral regions where measurements are made.

LITERATUR

ELLENBY, C. (1957). Some physiological aspects of the hatching mechanism of the potato-root eelworm, *Heterodera rostochiensis* Wollenweber. *Proc. Symposium "Insect and Foodplant", Wageningen* 1957, 95-102.

—— (1958 a). Root diffusates of *Solanum tuberosum* and *Digitalis purpurea*. *Nature, Lond.* **181**, 920-921.

—— (1958 b). Preliminary observations on the colorimetric assay of the hatching factor of the potato-root eelworm, *Heterodera rostochiensis* Wollenweber. *J. Helm.* **32**, 219-226.

ELLENBY, C. & GILBERT, A. B. (1957). Cardiotonic activity of the potato-root eelworm hatching factor. *Nature, Lond.* **180**, 1105-1106.

FENWICK, D. W. (1949). Investigations on the emergence of larvae from the cysts of the potato-root eelworm, *Heterodera rostochiensis*. I. Technique and variability. *J. Helm.* **23**, 157-170.

—— (1952). The bio-assay of potato-root diffusate. *Ann. appl. Biol.* **39**, 457-467.

FENWICK, D. W. & WIDDOWSON, E. (1958). The conduct of hatching tests on cysts of the potato-root eelworm *Heterodera rostochiensis* (Woll.). *J. Helm.* **32**, 125-134.

ROWSON, J. M. (1952). Studies in the genus *Digitalis*. I. The colorimetric estimation of digitoxin and of preparations of *Digitalis purpurea*. *J. Pharmacol.* **4**, 814-830.

—— (1955). Studies in the genus *Digitalis*. III. The extraction and evaluation of *Digitalis purpurea* leaf. *J. Pharmacol.* **7**, 924-931.

ROWSON, J. M. & DYER, F. J. (1952). Studies in the genus *Digitalis*. II. A comparison of the colorimetric and biological methods for the evaluation of *Digitalis purpurea*. *J. Pharmacol.* **4**, 831-843.

SEMBDNER, G. & SCHREIBER, K. (1960). Über die schlüpfaktive bzw. schlüpfhemmende Wirkung der Wurzeldiffusate verschiedener Pflanzen auf den Kartoffelnematoden, *Heterodera rostochiensis* Woll. 2. Mitt. über *Heterodera-Arten*. *Nematologica* (*Suppl. II*), 127-140.

WIDDOWSON, E. & WILTSHIRE, G. H. (1958). The potato-eelworm hatching factor. *Ann. appl. Biol.* **46**, 95-101.

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Nematologica 6 (1961): 25-36. Leiden, E. J. Brill

ZUR TAXONOMIE DER NEOTYLENCHIDEN

VON

I. ANDRÁSSY

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Drei neue Arten der Familie Neotylenchidae aus Ungarn werden beschrieben, u. zw. *Neotylenchus serpens*, *Nothotylenchus innuptus* und *N. antricolus* n. spp. Weiter beschäftigt sich der Verfasser mit der modifizierten Diagnose der Gattungen *Hexatylus* und *Neotylenchus*, sowie mit der als neu beschriebenen Gattung *Scytaleum* n. gen. Letztere Gattung wird für solche Arten der Neotylenchiden aufgestellt, dessen Ösophagus keinen abgesonderten Isthmus aufweist. Auch die Artenschlüssel der erwähnten drei Gattungen werden angegeben.

I. DREI NEUE ARTEN DER FAMILIE NEOTYLENCHIDAE THORNE, 1949

Neotylenchus serpens n. sp.

(Abb. 1 A-E)

Holotypus (♀): L = 1,21 mm; a = 60,0; b = 6,0; c = 29,5; V = 91,6%.

Körper sehr schlank, mit Ausnahme der beiden Enden ganz zylindrisch, schlangenartig. Kutikula dünn, an der Körpermitte 0,8-0,9 μ dick, Ringelung schwach. Seitenmembrane deutlich, bis zum Schwanzende reichend, etwa 1/4 so breit (5 μ) wie der Körper. Sie trägt 5 gut ausgeprägte Längsfelder (6 Furchen).

Kopfende nicht abgesetzt, verhältnismässig breit, vorn fast gerade abgestutzt. Die Ringelung lässt die Kutikula des Kopfes frei. Der Körper ist am Ende des Ösophagus fast dreimal so breit wie an der Basis des Kopfes. Der Kopf besitzt einen kaum kutikularisierten Versteifungsapparat („labial framework"). Mundstachel schlank, mittelmässig kutikularisiert, mit ovalen, schräg nach hinten gerichteten Basalknöpfen. Sein vorderer Abschnitt ist kürzer als der hintere. Die Stachellänge beträgt 8,5 μ, bzw. 1,2 Kopfbreiten. Die Mündung der dorsalen Ösophagusdrüse befindet sich kurz hinter den Knöpfen.

Ösophagus eigenartig geformt. Procorpus schlank mit schwach entwickelter, spindelförmig verlängerter Mittelanschwellung ohne Valvularapparat. Isthmus ebenfalls sehr schlank. Metacorpus (Endbulbus) verhältnismässig lang, am Ende mit einem fingerförmigen Dorsalfortsatz. Hinterer Abschnitt des Ösophagus (Isthmus + Metacorpus) fast anderthalbmal so lang wie der Procorpus. Drüsenkerne klein, nicht auffallend.

Kardia ausserordentlich klein, tropfenförmig. Exkretionsporus in der Höhe der Bulbusmitte liegend. Deiriden deutlich. Mitteldarm licht gefärbt, sehr wenig granuliert, Enddarm etwas länger als die anale Körperbreite.

Weibliche Gonade unpaarig, prävulvar, gestreckt. Vulva sehr weit hinten lie-

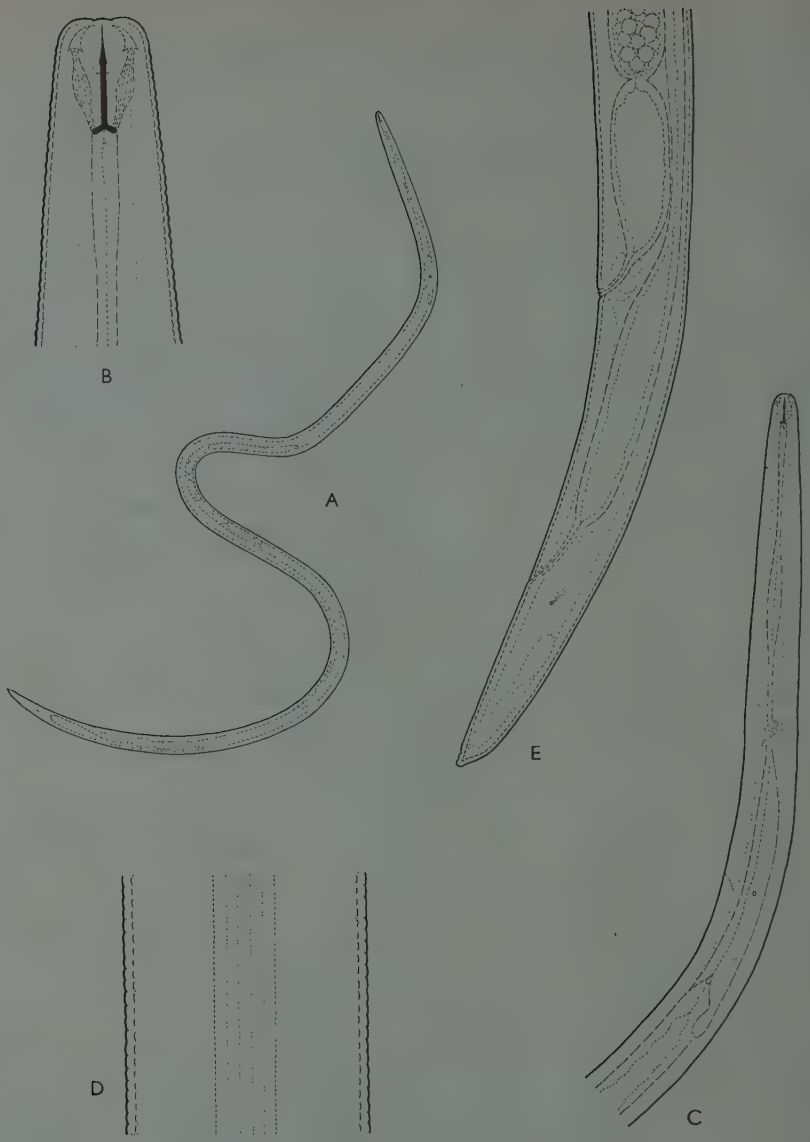

Abb. 1. *Neotylenchus serpens* n. sp. *A*: Habitusbild, 140 ×; *B*: Vorderende, 1600 ×; *C*: Ösophagusregion, 500 ×; *D*: Körperteil mit Seitenmembran, 1600 ×; *E*: Hinterkörper des Weibchens, 750 ×.

gend, mit kleinen, vorragenden Lippen. Vagina dünn, röhrenförmig, nach vorn gebogen. Spermatheka mit rundlichen Spermien; hinterer Uterusast fehlt.

Entfernung Vulva-Anus fast anderthalbmal so lang wie der Schwanz. Letzterer

ziemlich untersetzt, 3 Analbreiten lang, am Ende plötzlich verschmälert, dann sehr fein abgerundet. Phasmidien deutlich, kurz hinter der Analöffnung liegend. — Männchen unbekannt.

Kurze Diagnose: Eine sehr schlanke *Neotylenchus*-Art mit dünner, schwach geringelter Kutikula, 5 Längsfelder tragender Seitenmembran, breitem, nicht abgesetztem Kopf, länglichen Stachelknöpfen, sehr schlanker mittlerer Ösophagusanschwellung, langem, kleinen Dorsalfortsatz tragendem Endbulbus, unpaariger Gonade, weit hinten liegender Vulva, keinem hinteren Uterussack und ziemlich plumpem Schwanz. ♂ unbekannt.

Die neue Art unterscheidet sich durch den schlanken, schlangenartigen Habitus, die Schlankheit des Ösophagus und die Form des Schwanzes von allen bekannten Arten der Gattung *Neotylenchus*. Im Bau des hinteren Bulbus erinnert sie an *N. intermedius* (Christie, 1938) Thorne, 1941, *N. acutus* Thorne, 1941 und *N. dendrophilus* Rühm, 1956, weicht aber von ihnen durch die oben erwähnten Merkmale, ferner dadurch ab, dass der Schwanz kürzer ist als die Entfernung Vulva-Anus und die Seitenmembrane aus 5 Längsfeldern bestehen.

Holotypus: 1 Weibchen mit den oben erwähnten Körperausmassen. Paratypus: 1 juveniles Exemplar von demselben Fundort wie Holotypus.

Typischer Fundort: Bánkut im Bükk-Gebirge, Ungarn, Walderde. Gesammelt im Mai.

Nothotylenchus innuptus n. sp.

(Abb. 2 A-F)

♀: L = 0.47-0,49 mm; a = 25,2-28,7; b = 4,5-5,0; c = 7,3-8,3; V = 63,7-66,0%.

Körper klein, mässig schlank. Kutikula sehr dünn (0,7 μ), fein geringelt. Ringelbreite etwa 0,8 μ. Seitenmembrane deutlich, 1/4-1/5 der Körperbreite, mit 3 Längsfeldern (4 Furchen). Kopf abgesetzt, an seiner Basis schmäler als der nachfolgende Halsteil, mehr oder minder konisch, breiter als hoch, mit ungeringelter Kutikula. Versteifungsapparat tylenchoid, schwach. Körper am Proximalende des Ösophagus 2,7-2,9mal so breit wie an der Kopfbasis. Mundstachel 11,6-12,5 μ lang, 2-2,2mal so lang wie die Kopfbreite (an seinem Grund gemessen), bzw. 1/8-1/9 der Ösophaguslänge. Stachelknöpfe rundlich. Die Mündung der dorsalen Ösophagusdrüse befindet sich etwa 1/4 Stachellänge hinter den Knöpfen.

Vorderabschnitt des Ösophagus etwas länger als der hintere Abschnitt (sie verhalten sich wie 5 : 4; konstantes Merkmal!). Mittlere Ösophagusanschwellung länglich, etwa dreimal so lang wie breit, ohne Valvularapparat. Endbulbus mit drei Kernen, von denen der Dorsalkern auffallend gross ist (eine, für die Gattung *Nothotylenchus* charakteristische Erscheinung). Exkretionsporus knapp vor dem hinteren Bulbus. Deiriden vorhanden, Kardia scheibenförmig. Darm dicht granuliert, Enddarm so lang oder etwas länger als der anale Körperdurchmesser.

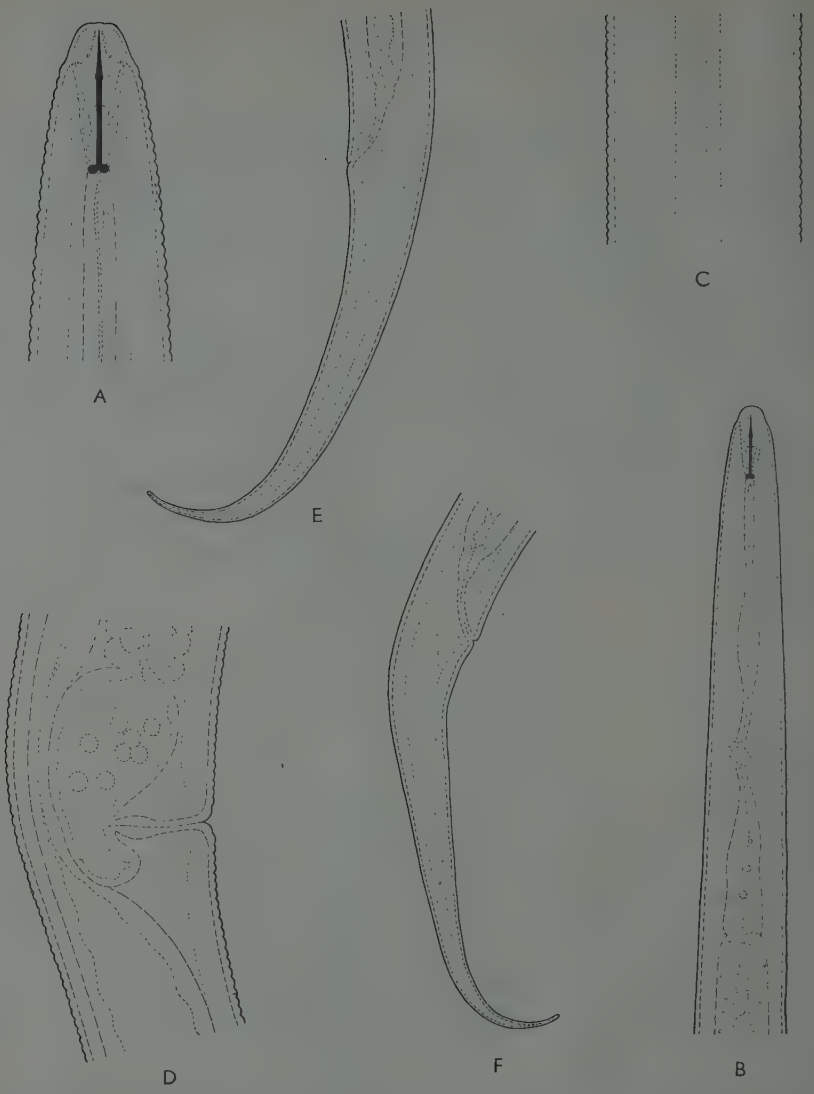

Abb. 2. *Nothotylenchus innuptus* n. sp. *A*: Vorderende, 1600 ×; *B*: Ösophagusregion, 750 ×; *C*: Körperabschnitt mit Seitenmembran, 1600 ×; *D*: Vulvagegend, 1600 ×; *E-F*: Hinterkörper verschiedener Weibchen, 1100 ×.

Weibliche Gonade unpaarig, prävulvar, 7,3-9,8mal so lang wie die mittlere Körperbreite. Die Vulvalippen sind nicht vorragend, der Körper verengt sich aber hinter der Vulva merklich. Die Körperbreite beträgt unmittelbar vor der Vulva 16,5-20 μ, kurz hinter derselben 13,5-15 μ und in der Höhe der Analöff-

nung 10-10,5 μ. Vagina gerade nach innen gerichtet, etwa halb so lang wie der betreffende Körperdurchmesser, schlank, bzw. am Proximalende etwas angeschwollen. Spermatheka mit kugeligen Spermien. Hinterer Uterusast sehr klein, kürzer als die halbe Körperbreite.

Entfernung Ösophagushinterende-Vulva im allgemeinen zweimal so lang wie der Ösophagus selbst, Entfernung Vulva-Anus 1,5-2mal länger als der Schwanz. Letzterer gleichmässig verjüngt, 5,6-6,2 Analbreiten lang, am Ende zugespitzt oder sehr fein abgerundet. Phasmidien nicht nachweisbar. — Männchen unbekannt.

Kurze Diagnose: Eine *Nothotylenchus*-Art mit abgesetztem Kopf, verhältnismässig langem Mundstachel, länglicher mittlerer Ösophagusanschwellung, sehr grossem Dorsalkern, verhältnismässig langem Procorpus, unpaariger, prävulvarer Gonade, kurzem Postvulvarschlauch, drei Längsfelder tragender Seitenmembrane und mittellangem, mehr oder weniger abgerundetem Schwanz. ♂ unbekannt.

Nothotylenchus innuptus n. sp. kann durch den abgesetzten Kopf, den verhältnismässig langen Mundstachel und die grosse Länge des vorderen Ösophagusabschnittes von sämtlichen Arten der Gattung unterschieden werden.

Holotypus: (♀) L = 0,48 mm; a = 27,4; b = 4,5; c = 8,2; V = 63,7%. Paratypi: 5 ♀ von demselben Fundort wie Holotypus.

Typischer Fundort: Budapest, Bezirk XI, aus Wiesenboden.

Nothotylenchus antricolus n. sp.

(Abb. 3 A-F)

♀: L = 0,41-0,55 mm; a = 44,6-52,7; b = 4,4-4,9; c = 6,7-6,9; V = 70,7-72,4%.

Kleine, schlanke Art. Kutikula ausserordentlich dünn, nur 0,5-0,6 μ an der Mitte des Körpers, kaum wahrnehmbar geringelt. Seitenmembrane deutlich, 1/4 so breit wie der Körper selbst, aus drei Längsfeldern (4 Furchen) bestehend. Kopf abgerundet, nicht abgesetzt mit ungeringelter Kutikula. Körper am Ende des Ösophagus 2,2mal so breit wie an der Basis des Kopfes. Mundstachel zart, 6,6-7,0 μ lang, 1,6-1.8mal so lang wie die Kopfbreite; Stachelknöpfe deutlich, oval.

Ösophagus sehr schlank, der hintere Abschnitt (Isthmus + Metacorpus) 1,4-1,5 mal so lang wie der vordere (vom Kopfende bis zum Ende des Procorpus). Procorpus ohne mittelbulbusartige Anschwellung, vollkommen zylindrisch, ebenso wie der ungewöhnlich verlängerte, hintere Bulbus. Isthmus verhältnismässig sehr lang, manchmal länger als der Metacorpus selbst. Exkretionsporus nicht weit vor dem Hinterbulbus. Darm und Leibeshöhle stark granuliert, Enddarm und Analöffnung ausserordentlich schwer nachweisbar oder völlig unerkennbar.

Entfernung Ösophagushinterende-Vulva 2,1-2,5mal so gross wie die Ösophaguslänge, Entfernung Vulva-Anus so lang oder etwas kürzer als der Schwanz.

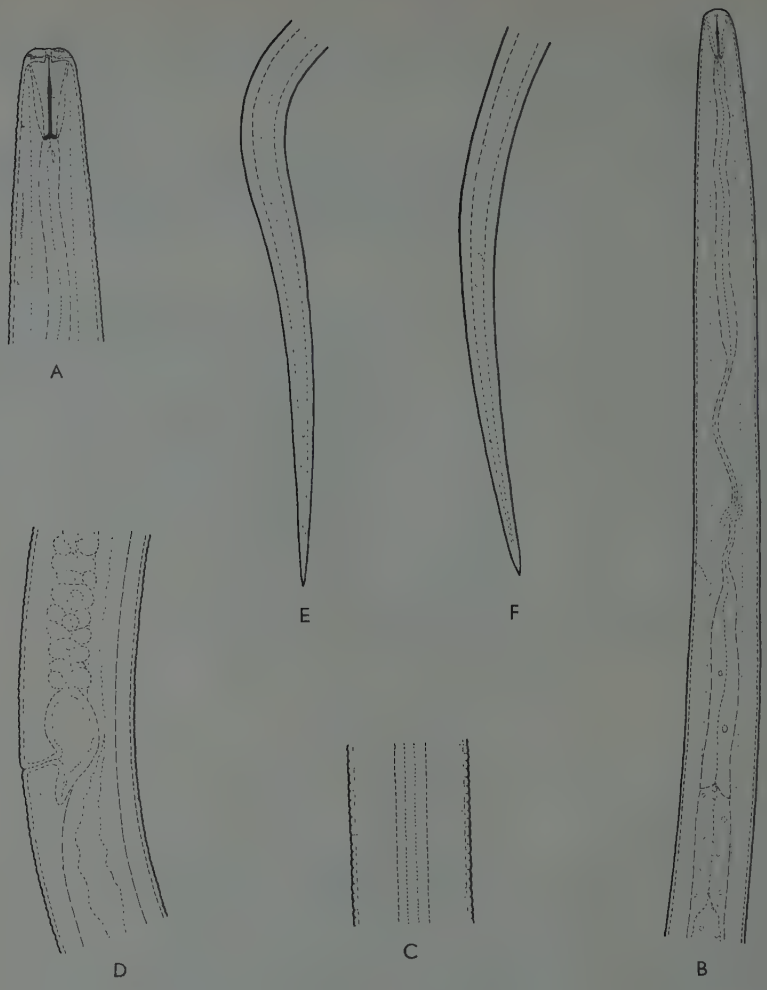

Abb. 3. *Nothotylenchus antricolus* n. sp. *A*: Vorderende, 1600 ×; *B*: Ösophagusregion, 1100 ×; *C*: Körperteil mit Seitenmembran, 1600 ×; *D*: Vulvagegend, 1600 ×; *E-F*: Hinterkörper von zwei Weibchen, je 750 ×.

Vagina schmal, röhrenförmig, etwa so lang wie 1/3 des entsprechenden Körperdurchmessers. Gonade unpaarig, prävulvar, 13-15mal so lang wie die mittlere Körperbreite. Postvulvarer Uterussack vorhanden aber sehr kurz, kleiner als die korrespondierende Breite des Körpers. Schwanz 9,6-10 Analbreiten lang, am Ende zugespitzt, aber nie fadenartig ausgezogen. Phasmidien undeutlich. — Männchen unbekannt.

Kurze Diagnose: Eine *Nothotylenchus*-Art mit folgenden charakteristischen

Merkmalen: Körper klein und schlank, Kutikula sehr dünn, Seitenmembrane mit 3 Längsfurchen, Ösophagus auffallend schlank, Procorpus und auch Metacorpus ganz zylindrisch, Enddarm und After undeutlich, hinterer Uterusteil klein und Schwanz verhältnismässig lang, zugespitzt. ♂ unbekannt.

Nothotylenchus antricolus n. sp. erinnert im Bau des Ösophagus stark an *N. cylindricollis* Thorne, 1941. Sein Körper ist aber kleiner und schlanker, der Schwanz bedeutend länger und der Postvulvarsack des Uterus kürzer als bei *cylindricollis*. Die neue Art weicht durch die Kombination ihrer Merkmale von den übrigen Arten der Gattung noch wesentlicher ab.

Holotypus: (♀) L = 0,49 mm; a = 48,7; b = 4,4; c = 6,7; V = 71,0%. Paratypi: 6 Weibchen von demselben Fundort wie Holotypus.

Typischer Fundort: Kölyuk-Höhle von Mánfa im Komitat Baranya, Südungarn, aus feuchtem, vermoderndem Holzstück. Leg.: Dr. I. Łoksa.

SCHLÜSSEL DER NOTHOTYLENCHUS-ARTEN

1(2) Kopf deutlich abgesetzt; Procorpus länger als der hintere Ösophagusabschnitt. — ♀: L = 0,47-0,49 mm; a = 25,2-28,7; b = 4,5-5,0; c = 7,3-8,3; V = 63,7-66,0 %. ♂ unbekannt. *innuptus* n. sp.

2(1) Kopf nicht abgesetzt; Procorpus höchstens so lang wie der hintere Ösophagusabschnitt, meistens aber viel kürzer.

3(8) Schwanzende fein abgerundet.

4(5) Mundstachel sehr klein, kürzer als die Kopfbreite; Männchen nicht bekannt. — ♀: L = 0,38-0,41 mm; a = 36,6-38,6; b = 4,1-4,3; c = 6,7-7,4; V = 67,4-67,8 %. ♂ unbekannt. *exiguus* Andrássy, 1958

5(4) Mundstachel länger als die Kopfbreite; Männchen bekannt.

6(7) Seitenmembrane mit 5 Längsfeldern; Bursa mindestens bis 1/3 der Schwanzlänge reichend. — ♀: L = 0,65 mm; a = 33; b = 5; c = 9; V = 71 %. ♂: L = 0,64 mm; a = 32; b = 6,4; c = 11. *affinis* Thorne, 1941

7(6) Seitenmembrane mit 3 Längsfeldern; Bursa kleiner, auch 1/4 der Schwanzlänge nicht erreichend. — ♀: L = 0,43 mm; a = 33,5; b = 4,4; c = 6,3; V = 66,7 %. ♂: L = 0,44 mm; a = 31,5; b = 4,5; c = 6,1. *loksai* Andrássy, 1959

8(3) Schwanzende zugespitzt.

9(12) Postvulvarer terusteil bedeutend länger als die betreffende Körperbreite.

10(11) Schwanzende stets ventral hakenartig gebogen; Bursa sehr klein, adanal. — ♀: L = 0,72-0,79 mm; a = 45,0-46,4; b = 5,5-7,0; c = 9,3-10,4; V = 72,2-73,3 %. ♂: L = 0,61-0,67 mm; a = 43,6-57,3; b = 7,0-7,2; c = 8,8-9,1. *danubialis* Andrássy, 1960

11(10) Schwanzende fast gerade; Bursa gross, die halbe Schwanzlänge überschreitend. — ♀: L = 0,8-1,1 mm; a = 33,0-51,5; b = 6,2-6,5; c = 9,6-16,5; V = 79-80 %. ♂: L = 0,68-0,70 mm; a = 35,0-36,6; b = 5,1-6,5; c = 9,5-9,9. *acris* Thorne, 1941

12(9) Postvulvarer Uterusteil klein, höchstens so lang wie die betreffende Körperbreite (meist aber viel kürzer).

13(16) Vorderer Ösophagusabschnitt zylindrisch, ohne mittelbulbusartige Anschwellung, Endbulbus ungewöhnlich verlängert, ganz zylindrisch.

14(15) Schwanz des Weibchens etwa 10 Analbreiten lang; postvulvarer Uterusteil kürzer als 1/2 der Körperbreite; sehr schlanke Art. — ♀: L = 0,41-0,55 mm; a = 44,6-52,7; b = 4,4-4,9; c = 6,7-6,8; V = 70,7-72,4 %. ♂ unbekannt. *antricolus* n. sp.

15(14) Schwanz des Weibchens nur etwa 4 Analbreiten lang; postvulvarer Uterusteil so lang wie die Körperbreite; Körper nicht so schlank. — ♀: 0,7 mm; a = 34; b = 5; c = 17; V = 90 %. ♂: L = 0,9 mm; a = 40; b = 5; c = 12. *cylindricollis* Thorne, 1941

16(13) Vorderer Ösophagusabschnitt mit mittelbulbusartiger Anschwellung, Endbulbus normal, flaschenförmig.

17(18) Endbulbus asymmetrisch, d.h. dorsal nach hinten verlängert; Vulvalippen auffallend kutiku-

larisiert; Schwanz mit kleinem Endzapfen. — ♀: L = 0,495 mm; a = 44,0; b = 4,0; c = 10,0; V = 75,8%. ♂ unbekannt. *thornei* Andrássy, 1958

18(17) Endbulbus symmetrisch, dorsal nach hinten nicht verlängert; Vulvalippen normal kutikularisiert; Schwanz ohne Endzapfen. — ♀: L = 0,72-0,98 mm; a = 24,0-24,5; b = 5,3-5,8; c = 9,7-11,8; V = 81,1-84,5 %. ♂: L = 0,65-0,82 mm; a = 34,1-35,2; b = 4,8-5,9; c = 6,9-7,1. *drymocolus* Rühm, 1956

II. ÜBER DIE GATTUNGEN HEXATYLUS GOODEY, 1926, SCYTALEUM N. GEN. UND NEOTYLENCHUS STEINER, 1931

Thorne (1941) reihte in seiner ausgezeichneten Arbeit die Tylenchiden-Arten, deren Ösophagus keinen echten, Valvularapparat tragenden Mittelbulbus besitzt, in die Unterfamilien Neotylenchinae Thorne, 1941, Paurodontinae Thorne, 1941 und Nothotylenchinae Thorne, 1941. Diese drei Unterfamilien zog er später (1949) in die neu aufgestellte Familie Neotylenchidae Thorne, 1949 zusammen. Drei Jahre später (1952) trennte Skarbilovich [1]) die Gattungen *Hexatylus* und *Deladenus* von den anderen Gattungen der Neotylenchinen ab und stellte für sie die neue Unterfamilie Hexatylinae Skarbilovich, 1952 auf. Gleichzeitig modifizierte der sowjetische Verfasser die Diagnose der Gattung *Hexatylus* und reihte die von Thorne in die Gattung *Neotylenchus* gestellte Art *H. coprophagus* Goodey, 1938 wieder in das Genus *Hexatylus* zurück. Zusammen mit der von Skarbilovich beschriebenen Spezies *H. vigissi* Skarbilovich, 1952 enthielt die Gattung folgende drei Arten: *Hexatylus viviparus, H. coprophagus* und *H. vigissi.* Seither wurden von Meyl (1954) vier, von Amakhanov (1958) noch zwei Arten unter dem Gattungsnamen *Hexatylus* beschrieben.

Die Gattung erwies sich aber inzwischen äusserst heterogen, da die ihr zugehörigen Arten in zwei, voneinander scharf abgesonderte Gruppen eingereiht werden können. Bei einem Teil der Arten geht der Metacorpus des Ösophagus nämlich ohne Grenze in den Darm über, beim anderen hingegen ist er vom Darm scharf abgegrenzt. Auf Grund dieses taxonomisch bedeutenden Unterscheidungsmerkmales weichen die beiden Gruppen voneinander so stark ab, dass sie in ein und derselben Gattung weiter nicht stehen gelassen werden dürfen. Zur Gattung *Hexatylus* gehören also nur die Arten, deren Ösophagus in den Mitteldarm allmählich übergeht (*H. viviparus, H. boettgeri, H. brevicaudatus* und *H. dipapillatus*).

Die übrigen, unter dem Gattungsnamen *Hexatylus* beschriebenen Arten dürften in die Gattung *Neotylenchus* übertragen werden. Zwei Arten von ihnen — *H. vigissi* und *H. skarbilowiezae* — bilden aber zusammen mit zwei, unter dem Gattungsnamen *Neotylenchus* bekannt gewordenen Arten *N. italicus* und *N. thornei* eine gut abgegrenzte Gruppe, die nun auch von den „echten" Neotylenchen abweicht. Der Ösophagus dieser Arten besitzt nämlich keinen Isthmus, d.h. der vollkommen zylindrische Procorpus geht direkt — also ohne irgend eine Zwischenpartie — in den Metacorpus über. Ich halte es für begründet und zweckmässig,

[1]) Ich übertrage die cyrillischen Namen und Wörter nach dem in „Bulletin of Zoological Nomenclature" (Band 11, Heft 1, 1955) veröffentlichten Vorschlag.

diese Arten sowohl von den *Hexatylus*- als auch von den *Neotylenchus*-Arten abzutrennen und sie in Form einer selbständigen Gattung — *Scytaleum* n. gen. — zusammenzufassen.

Nachstehend gebe ich die Diagnosen und Artenschlüssel der umgebildeten Gattungen *Hexatylus* und *Neotylenchus,* sowie der neuen Gattung *Scytaleum* an.

Gattung *Hexatylus* Goodey, 1926

Diagnose: Kopf oktagonal. Mundstachel kurz, nicht länger als die Kopfbreite, mit deutlichen Knöpfen. Procorpus des Ösophagus plump ohne bulbusartige Anschwellung und Valvularapparat; Isthmus vom Pro- und Metacorpus abgesondert, aber sehr kurz. Hinterer Ösophagusteil ohne scharfe Grenze in den Darm übergehend. Ösophagusdrüsen im Metacorpus. Kardia fehlt. Gonade prävulvar ohne hinteren Uterussack. Schwanz kurz, mehr oder weniger zugespitzt, Phasmidien undeutlich. Männchen unbekannt.

Saprobionte und mycobionte Arten.

Typische Art: *Hexatylus viviparus* Goodey, 1926. — 4 Arten:

H. boettgeri Meyl, 1954
H. brevicaudatus Meyl, 1954
H. dipapillatus Meyl, 1954
H. viviparus Goodey, 1926 — Syn.: *Anguillulina vivipara* (Goodey, 1926) Schneider, 1939; *Iotonchium viviparum* (Goodey, 1926) Filipjev & Schuurmans Stekhoven, 1941.

SCHLÜSSEL DER HEXATYLUS-ARTEN

1(4) Schwanz durch eine Einschnürung deutlich in zwei Abschnitte geteilt.
2(3) Körper in der Mitte deutlich aufgetrieben; Vorderkörper zylindrisch, nach vorn kaum verschmälert; Kutikula geringelt; distaler Schwanzteil beweglich, zitronenförmig. — ♀: L = 0,76-1,03 mm; a = 16,9-22,8; b = 7,6-9,1; c = 25,0-31,3; V = 86,9-89,0%. ♂ unbekannt. *boettgeri* Meyl, 1954
3(2) Körper in der Mitte nicht aufgetrieben; Vorderkörper nach vorn stark verschmälert; Kutikula ungeringelt; distaler Schwanzteil nicht beweglich. — ♀: L = 1,13 mm; a = 20,2; b = 9,8; c = 31,2; V = 88,0%. ♂ unbekannt. *brevicaudatus* Meyl, 1954
4(1) Schwanz ohne Einschnürung, mehr oder minder konisch.
5(6) Schwanz von der Mitte ab zylindrisch verlaufend, mit zwei Paare subdorsaler Papillen; Kutikula glatt. — ♀: L = 0,76-0,85 mm; a = 14,1-17,6; b = 6.5-8,5; c = 9,9-11,4; V = 80-83%. ♂ unbekannt. *dipapillatus* Meyl, 1954
6(7) Schwanz regelmässig konisch, ohne deutliche Papillen; Kutikula geringelt. — ♀: L = 0,76-1,50 mm; a = 15-35; b = 5-7; c = 17-20; V = 81-89%. ♂ unbekannt. *viviparus* Goodey, 1926

Gattung *Scytaleum* n. gen.

Diagnose: Stachel deutlich geknöpft. Ösophagus nur aus Procorpus und Metacorpus bestehend. Isthmus völlig in Procorpus verschmolzen, dicht abgesondert. Vorderer Ösophagusabschnitt ganz zylindrisch, ohne mittelbulbusartige Anschwellung und Valvularapparat. Hinterbulbus vom Darm scharf abgetrennt, am Ende lobus-artig. Ösophagusdrüsen im Metacorpus. Kardia vorhanden aber sehr klein. Gonade prävulvar, Bursa schwanzumfassend.

Terrikole und saprophile Arten. Die Gattung scheint einen Übergang zwischen den Genera *Hexatylus* und *Neotylenchus* zu bilden.

Typische Art: *Scytaleum vigissi* (Skarbilovich, 1952) n. comb. — 4 Arten:

S. italicum (Meyl, 1954) n. comb. — Syn.: *Neotylenchus italicus* Meyl, 1954
S. skarbilowiezae (Amakhanov, 1958) n. comb. — Syn.: *Hexatylus skarbilowiezae* Amakhanov, 1958
S. thornei (Meyl, 1954) n. comb. — Syn.: *Neotylenchus thornei* Meyl, 1954
S. vigissi (Skarbilovich, 1952) n. comb. — Syn.: *Hexatylus vigissi* Skarbilovich, 1952.

SCHLÜSSEL DER SCYTALEUM-ARTEN

1(2) Schwanz kuppelförmig mit aufgesetztem Endzapfen; Stachel kürzer als die Kopfbreite. — ♀: L = 1,45 mm; a = 20,4; b = 12,1; c = 34,5; V = 84,4%. ♂ unbekannt.
italicum (Meyl, 1954) n. comb.

2(1) Schwanz mehr oder minder konisch, ohne aufgesetzten Endzapfen; Stachel so lang oder länger als die Kopfbreite.

3(4) Endbulbus etwa ½ der Ösophaguslänge einnehmend. — ♀: L = 0,59-0,62 mm; a = 28,0-29,7; b = 3,0-3,7; c = 12,0-13,2; V = 83,0-84,1%. ♂: L = 0,45-0,47 mm; a = 30-31; b = 2,6-2,8; c = 17-18. *skarbilowiezae* (Amakhanov, 1956) n. comb.

4(3) Endbulbus nur etwa 1/3 der Ösophaguslänge einnehmend.

5(6) Schwanz des Weibchens im letzten Fünftel zylindrisch, am Ende abgerundet; Vulva auffallend kutikularisiert. — ♀: L = 0,74-0,81 mm; a = 27,0-32,0; b = 4,3-5,4; c = 15,0-15,5; V = 78,3-78,7 %. ♂ unbekannt. *thornei* (Meyl, 1954) n. comb.

6(5) Schwanz des Weibchens gleichmässig konisch, zugespitzt; Vulva normal kutikularisiert. — ♀: L = 0,89-1,33 mm; a = 23,4-34,5; b = 6,2-6,8; c = 12,7-16,5; V = 77,6-93,2%. ♂: L = 0,88-1,22 mm; a = 41,0-48,4; b = 6,5-7,6; c = 24,6-30,3.
vigissi (Skarbilovich, 1952) n. comb.

Gattung *Neotylenchus* Steiner, 1931

Diagnose: Kopf oktagonal. Mundstachel gewöhnlich deutlich geknöpft. Procorpus zumeist ohne mittelbulbusartige Anschwellung, vom Metacorpus durch einen stets vorhandenen, schmäleren Isthmus abgetrennt. Endbulbus vom Darm scharf abgesondert, manchmal ein wenig dorsal verlängert. Ösophagusdrüsen im Metacorpus. Kardia vorhanden aber sehr klein. Gonade prävulvar mit oder ohne Postvulvarteil. Schwanz kurz oder mässig lang. Phasmidien nur bei einer Art deutlich. Bursa schwanzumfassend oder fast bis zur Spitze des Schwanzes reichend (fehlt bei *N. dendrophilus*).

Terrikole, saprophile oder an Pflanzenwurzeln gebundene Arten.

Eine nicht ganz homogene Gattung. Die nachstehend aufgezählten Arten können morphologisch in drei Gruppen eingereiht werden. Es sind dies: a) Ösophagus ohne mittelbulbusartige Anschwellung, Bursa schwanzumfassend (*N. abulbosus, N. beljaevae, N. consobrinus, N. coprophagus, N. intermedius, N. latus* und *N. obesus*); b) Ösophagus mit mittelbulbusartiger Anschwellung, Bursa vor dem Schwanzende endigend (*N. arcuatus, N. serpens*); c) Ösophagus mit mittelbulbusartiger Anschwellung, Bursa und Gubernakulum nicht vorhanden (*N. dendrophilus*).

Typische Art: *Neotylenchus abulbosus* Steiner, 1931. — 11 Arten:

N. abulbosus Steiner, 1931 — Syn.: *Hexatylus abulbosus* (Steiner, 1931) Goodey, 1933; *Anguillulina abulbosa* (Steiner, 1931) Schneider, 1939; *Iotonchium abulbosum* (Steiner, 1931) Filipjev & Schuurmans Stekhoven, 1941

N. acutus Thorne, 1941 — Syn.: *Neotylenchus apiculatus* Thorne, 1941 lapsus pro *N. acutus*

N. arcuatus Thorne, 1941

N. beljaevae (Amakhanov, 1958) n. comb. — Syn.: *Hexatylus beljaevae* Amakhanov, 1958

N. consobrinus (de Man, 1907) Filipjev, 1936 — Syn.: *Tylenchus consobrinus* de Man, 1907; *exatylus consobrinus* (de Man, 1907) Goodey, 1932; *Anguillulina consobrina* (de Man, 1907) chneider, 1939; *Iotonchium consobrinum* (de Man, 1907) Filipjev & Schuurmans Stekhoven, 1941

N. coprophagus (Goodey, 1938) Thorne, 1941 — Syn.: *Hexatylus coprophagus* Goodey, 1938; *otonchium coprophagum* (Goodey, 1938) Filipjev & Schuurmans Stekhoven, 1941

N. dendrophilus Rühm, 1956

N. intermedius (Christie, 1938) Thorne, 1941 — Syn.: *Hexatylus intermedius* Christie, 1938; *otonchium intermedium* (Christie, 1938) Filipjev & Schuurmans Stekhoven, 1941

N. latus Thorne, 1935 — Syn.: *Iotonchium latum* (Thorne, 1935) Filipjev & Schuurmans Stekhoven, 1941

N. obesus Thorne, 1934 — Syn.: *Iotonchium obesum* (Thorne, 1934) Filipjev & Schuurmans Stekhoven, 1941

N. serpens n. sp.

SCHLÜSSEL DER NEOTYLENCHUS-ARTEN

1(12) Ösophagusbulbus dorsal nach hinten verlängert, lappen-artig.

2(7) Schwanz des Weibchens keilartig scharf zugespitzt.

3(4) Vorderer Ösophagusabschnitt mit bulbusartiger, länglicher Anschwellung; Bursa fehlt; kleine Art. — ♀: L = 0,26-0,63 mm; a = 10,9-19,7; b = ?; c = 6,5-13,7; V = 76,0-87,3 %. ♂: L = 0,41-0,62 mm; a = 25,8-26,0; b = ?; c = 9,8-12,2.
dendrophilus Rühm, 1956

4(3) Vorderer Ösophagusabschnitt ohne bulbusartige Anschwellung; Bursa vorhanden; grössere Arten, über 0,8 mm.

5(6) Isthmus sehr kurz, kürzer als ½ der korresp. Körperbreite; Eizellen in mehrere Reihen geordnet; Exkretionsporus am Ende des Ösophagus. — ♀: L = 0,88-1,45 mm; a = 18,5-27,0; b = 6,3-7,0; c = 8,0-10,4; V = 82-86 %. ♂ unbekannt.
coprophagus (Goodey, 1938) Thorne, 1941

6(5) Isthmus etwa so lang wie die korresp. Körperbreite; Eizellen in eine einzige Reihe geordnet; Exkretionsporus vor dem Endbulbus. — ♀: L = 0,8 mm; a = 26; b = 4,4; c = 9; V = 83 %. ♂ unbekannt. *acutus* Thorne, 1941

7(2) Schwanz des Weibchens mehr oder weniger abgerundet, nie scharf zugespitzt.

8(9) Körper auffallend schlank (a = 60); Phasmidien deutlich. — ♀: L = 1,21 mm; a = 60; b = 6; c = 29,5; V = 91,6 %. ♂ unbekannt. *serpens* n. sp.

9(8) Körper nicht so schlank (a kleiner als 40); Phasmidien undeutlich.

10(11) Mundstachel bedeutend kürzer als die Kopfbreite; Schwanz des Weibchens fast zylindrisch, gerade, breit abgerundet; Bursa schwanzumfassend. — ♀: L = 1,46 mm; a = 36; b = 7; c = 21; V = 88-94 %. ♂: L = 1,21 mm; a = 35; b = 8; c = 21.
intermedius (Christie, 1938) Thorne, 1941

11(10) Mundstachel länger als die Kopfbreite; Schwanz des Weibchens ± konisch, ventral gebogen, fein abgerundet; Bursa lässt das Schwanzende frei. — ♀: L = 1,1 mm; a = 15; b = 9; c = 22,5; V = 88 %. ♂: L = 1,1 mm; a = 39; b = 6,3; c = 17.
arcuatus Thorne, 1941

12(1) Ösophagusbulbus dorsal nicht verlängert.

13(14) Postvulvarer Uterusast vorhanden. — ♀: L = 0,7-1,1 mm; a = 17; b = 6,3; c = 18; V = 85 %. ♂: L = 0,8 mm; a = 31; b = 4,7; c = 20. *latus* Thorne, 1935

14(13) Postvulvarer Uterusast fehlt.

15(16) Schwanz des Weibchens fast zylindrisch, am Ende breit abgerundet mit einem kleinen aufgesetzten Zapfen. — ♀: L = 0,73-0,76 mm; a = 19,0-20,4; b = 4,9-5,1; c = 18,0-18,8; V = 87-88 %. ♂ unbekannt. *beljaevae* (Amakhanov, 1958) n. comb.

16(15) Schwanz des Weibchens konisch, zugespitzt ohne aufgesetzten Zapfen.

17(18) Vulva sehr weit hinten liegend; postvulvarer Körperteil nur etwa so lang wie die an der Vulva gemessene Körperbreite. — ♀: L = 0,7-0,9 mm; a = 10-16; b = 5-6; c = 20-50; V = 95-99 %. ♂ unbekannt. *obesus* Thorne, 1934

18(17) Vulva nicht so weit hinten liegend; postvulvarer Körperteil bedeutend länger.

19(20) Mundstachel kürzer als die Kopfbreite: Männchen mit fehlendem Stachel und reduzierten

Spikula. — ♀: L = 0,69-1,17 mm; a = 20,8-31,3; b = 4,0-5,5; c = 9,5-16,1; V = 85-89 %. ♂: L = 0,74 mm; a = 39; b = 11,2; c = 20. *abulbosus* Steiner, 1931

20(19) Mundstachel so lang oder länger als die Kopfbreite; Männchen mit Mundstachel und normal entwickelten Spikula. — ♀: L = 0,96-1,24 mm; a = 27-36; b = 5,7-7,3; c = 19-23; V = 89-90 %. ♂: L = 0,83-0,98 mm; a = 36-45; b = 6,3-7,7; c = 17,5-23,0. *consobrinus* (de Man, 1907) Filipjev, 1936

SUMMARY

The taxonomy of the Neotylenchidae

In the first part of the article, three new species of the family Neotylenchidae are described, two of them found in soil, and the third one (*antricolus*) in a mountain cave. They are characterised as follows: *Neotylenchus serpens* n. sp.: body very slender, lateral fields with six incisures, spear knobs oval, esophagus with slender middle swelling, terminal bulb lobed, postvulvar uterine branch absent, tail somewhat subdigitate, phasmids easily visible. *Nothotylenchus innuptus* n. sp.: head offset, stylet long, middle bulb elongated, procorpus longer than isthmus and metacorpus together, uterus with small posterior branch, tail long, ⌓ finely rounded terminally. *Nothotylenchus antricolus* n. sp.: body slender, cuticle exceedingly thin, pro- and metacorpus completely cylindrical, rectum and anus usually invisible, posterior uterine branch short, tail elongate, terminus acute. A key to *Nothotylenchus* species is given.

The second part contains the emended diagnostic characters of the genera *Hexatylus* and *Neotylenchus,* and establishes the new genus *Scytaleum* n. gen. for those species of *Hexatylus* and *Neotylenchus* the esophagus of which consists of procorpus and metacorpus only (without an isthmus). Keys to the species of these three genera are given.

SCHRIFTTUM

AMAKHANOV, Sh. A. (1958). *Sem novykh vidov nematod.* In: Rab. Gelmintol. 80 let akad. K. I. Skrjabina, Moskva, 42-49.

ANDRÁSSY, I. (1958). Erd- und Süsswassernematoden aus Bulgarien. *Acta Zool. Hung.* **4**, 1-88.

—— (1959). Freilebende Nematoden aus Rumänien. *Ann. Univ. Sci. Budapest.* **2**, 3-27.

—— (1960). Nematoden aus dem Psammon der Landungsmolen der Donau zwischen Budapest und Mohács. *Arch. Hydrobiol.* im Druck.

CHRISTIE, J. R. (1938). Two nematodes associated with decaying citrus fruit. *Proc. helm. Soc. Wash.,* **5**, 29-33.

GOODEY, T. (1926). Hexatylus viviparus gen. et sp. nov., a nematode found in a diseased potato tuber, *J. Helm.* **4**, 27-30.

—— (1926). A further note on Hexatylus viviparus Goodey, 1926. *J. Helminth.* **4**, 183-184.

—— (1938). Observations on two free-living nematodes, Hexatylus coprophagus n. sp. and H. consobrinus (de Man, 1906) Goodey, 1932. *Livro Jub. Prof. Travassos, Rio de Janeiro,* **3**, 179-182.

MEYL, A. H. (1954). Die Nematodenfauna höherer Pilze in Laub- und Nadelwäldern zwischen Braunschweig und dem Harz. *Mycopathol. Mycol. appl.* **7**, 1-80.

—— (1954). Die bisher in Italien gefundenen freilebenden Erd- und Süsswasser-Nematoden. *Arch. zool. Ital.* **39**, 161-264.

RÜHM, W. (1956). Die Nematoden der Ipiden. *Parasitol. SchrReihe* **6**, 1-437.

SKARBILOVICH, T. S. (1952). Analiz vidov roda Hexatylus Goodey, 1926. *Trudy Gelmintol. Lab.* **6**, 370-375.

STEINER, G. (1931). Neotylenchus abulbosus n. g., n. sp. (Tylenchidae, Nematoda), the causal agent of a new nematosis of various crop plants. *J. Wash. Acad. Sci.* **21**, 536-538.

—— (1932). The male of the nematode species Neotylenchus abulbosus Steiner, and its sexual dimorphism. *J. Wash. Acad. Sci.* **22**, 482-484.

THORNE, G. (1941). Some nematodes of the family Tylenchidae which do not possess a valvular median esophageal bulb. *The Great Basin Naturalist,* **2**, 37-85.

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EINE NEUE ART DER SELTENEN NEMATODEN-GATTUNG TRIPLONCHIUM COBB, 1920

VON

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Triplonchium pileatum n. sp. wird aus Ungarn beschrieben. Ihre wichtigsten Kennzeichen sind der kappenartig abgesetzte Kopf, die eingesenkten Lippenpapillen, die weit vorn liegenden Seitenorgane, das stark kutikularisierte Rektum und der feinere Bau des Spikularapparates.

Die Arten der von Cobb im Jahre 1920 aufgestellten Gattung *Triplonchium* bilden einen Übergang zwischen den freilebenden Dorylaimiden und den im Larvenstadium schon parasitischen Mermithiden. Obwohl die *Triplonchium*-Arten noch eine freilebende Lebensweise führen — alle von ihnen sind Erdbewohner — und eher an den Dorylaimiden erinnern, weisen sie bereits einige Mermithiden-artige Merkmale auf. Ihre Biologie und Ökologie ist fast vollkommen unbekannt. Da unsere Kenntnisse bezüglich ihrer Morphologie gleichfalls vieler Ergänzungen bedürfen, halte ich es für angezeigt, die sich in meiner Präparatensammlung befindliche, anscheinend neue *Triplonchium*-Art nachstehend ausführlich zu besprechen.

Triplonchium pileatum n. sp.

(Abb. 1 A-E)

Holotypus (♀): L = 1,19 mm; a = 28,3; b = 6,4; c = 29,1; V = 55,0%.
Allotypus (♂): L = 1,23 mm; a = 26,3; b = 5,2; c = 28,4.

Kutikula 1,4 μ dick, ausserordentlich fein geringelt, Ringelung auch durch Immersion kaum wahrnehmbar. Der kappenartige, vorn breit abgerundete Kopf ist stark abgesetzt, bzw. vom nachstehenden Körperteil durch eine tiefe Ringfurche scharf abgesondert. Er ist 17 μ breit, dreimal so breit wie hoch. Die Lippenpapillen sind etwas eingesenkt und nie vorragend, wie bei den anderen Arten der Gattung. Körper am Ende des Ösophagus, 2,1mal (♀) bzw. 2,5mal (♂) so breit wie an der Basis der Kopfes.

Seitenorgane kelchförmig mit ovaler Öffnung, 1/3 so breit wie die betreffende Körperbreite. Sie liegen verhältnismässig sehr weit vorn, u.zw. vor dem Distalende des Mundstachels. Sensillae deutlich, breiter und länger als die Seitenorgane selbst. Der für die Gattung charakteristisch geformte Mundstachel ist 16,0-17,5 μ lang, d.h. etwa so lang wie der Kopfdurchmesser; er trägt deutliche Basalknöpfe.

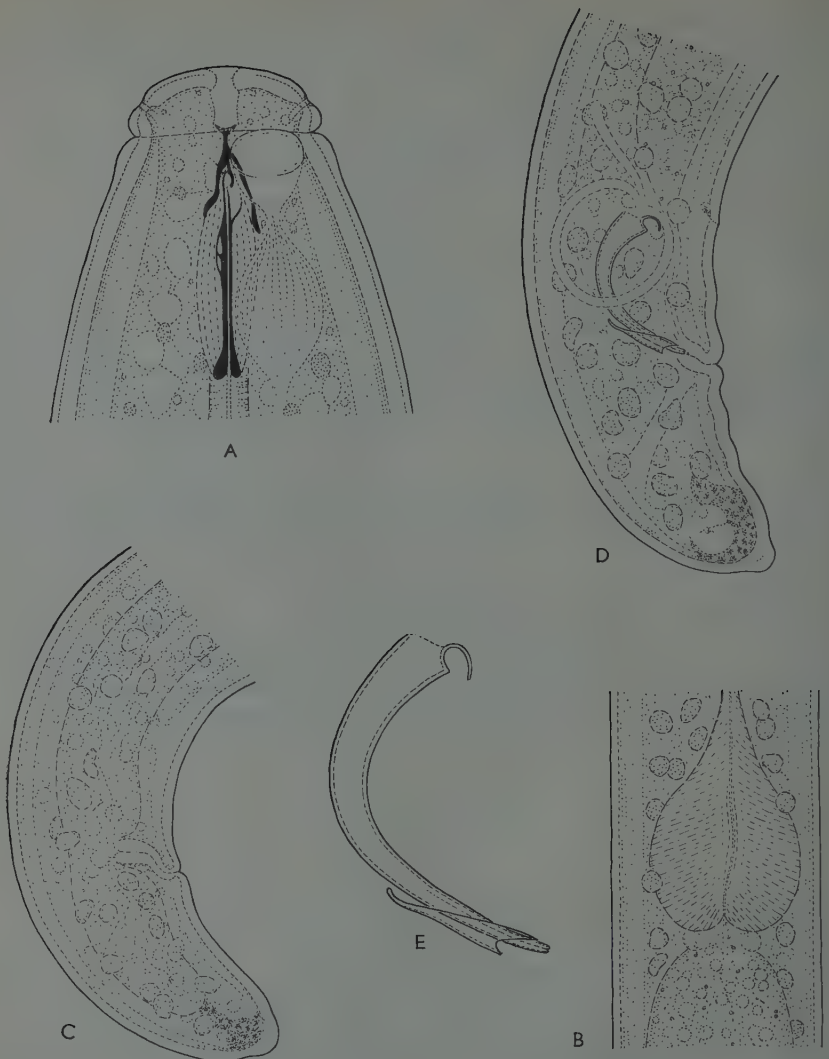

Abb. 1. *Triplonchium pileatum* n. sp. A: Vorderende, 1600 ×; B: Region des proximalen Ösophagusendes, 700 ×; C: Hinterkörper des Weibchens, 700 ×; D: Hinterkörper des Männchens, 700 ×; E: Spikularapparat, 1600 ×.

Akzessorischer Stachelapparat kompliziert gebaut, in Seitenansicht mit drei nach hinten gerichteten Fortsätzen versehen.

Ösophagus schlank, röhrenförmig, in dem letzteren Fünftel (82%) erweitert; Endanschwellung birnenförmig, 40 μ lang. Kardia undeutlich. Leibeshöhle und Mitteldarm mit zahlreichen Blasen von verschiedener Form und Grösse. Mittel-

darm sehr schwach konturiert, Enddarm dagegen auffallend kutikularisiert, vom Darm scharf abgesondert, beim Weibchen 17 μ lang, kürzer als 1/2 der analen Körperbreite. Der postanale Darmblindsack dringt tief in das Lumen des Schwanzes ein. Ein Prärektum fehlt.

Entfernung Ösophagushinterende-Vulva 2,5 mal so lang wie der Ösophagus selbst. Vulva querstehend, Vagina stark kutikularisiert, etwa 1/2 so lang wie die korrespondierende Körperbreite. Weibliche und männliche Gonaden paarig, Uterus lang. Spikula stark gebogen, 36 μ lang, distal allmählich verschmälert, aber nicht zugespitzt, proximal mit je einem sehr dünnen, ventral stark gebogenen, aufgesetzten Fortsatz versehen. Gubernakulum 13 μ lang. Spiralmuskulatur des Spikularapparates gut entwickelt. In der Höhe des proximalen Spikulumendes befindet sich ein einziges Präanalorgan. Postanalpapillen und Schwanzkanälchen nicht vorhanden.

Schwanz bei beiden Geschlechtern ähnlich, ventral schwach gebogen, 1,2-1,3 Analbreiten lang. Im Schwanzlumen befindet sich ein terminales, stark lichtbrechendes, gelbliches Gewebe.

Kurze Diagnose: Eine *Triplonchium*-Art mit folgenden Besonderheiten: Kutikula sehr fein geringelt, Kopf kappenartig scharf abgesetzt, Lippenpapillen eingesenkt, Seitenorgane weit vorn liegend, Gonaden paarig, Spikula und Gubernakulum charakteristisch gebaut, Enddarm stark kutikularisiert, Darmblindsack lang, ein einziges Präanalorgan vorhanden, Schwanz ohne Papillen.

Es sind bis heute drei Arten der Gattung *Triplonchium* Cobb, 1920 bekannt, und zwar *T. cylindricum* Cobb, 1920, *T. minor* Thorne, 1939 und *T. bulgaricum* Andrássy, 1958. Alle diese Arten und auch die oben beschriebene neue Art stehen in morphologischer Hinsicht einander sehr nahe, können aber durch gewisse Merkmale voneinander leicht abgetrennt werden (Tab. 1). Die Hauptunterscheidungsmerkmale von *T. pileatum* n. sp. sind der kappenartige, scharf abgesetzte Kopf, die nicht vorragenden Lippenpapillen, die vorn liegenden Seitenorgane und die feinere Organisation des Spikularapparates.

Es sei bemerkt, dass die von Thorne gefundenen *T. cylindricum*-Exemplare (besonders die in dem Staat Utah gesammelten) in gewissen Merkmalen von der typischen Art abweichen. Schon Thorne (1939) betonte, dass seine Tiere bedeutend grösser sind und einen längeren und schlankeren Schwanz besitzen. Thorne konnte ferner ein deutliches Präanalorgan feststellen, während die Typenexemplare von Cobb kein Präanalorgan besitzen (Cobb sah nur 1 oder 2 schwer erkennbare Papillen unmittelbar vor dem Anus). Auf Grund dieser Unterscheidungen ist es nicht ausgeschlossen, dass die Exemplare von Thorne zu einer anderen, bisher unbenannten Spezies gehören.

Holotypus (Weibchen) und Allotypus (Männchen) in der Sammlung des Verfassers.

Typischer Fundort: Alsógöd, Mittel-Nordungarn, aus sandigem Waldboden eines kleinen Donauinsels.

TABELLE I

Unterscheidungstabelle der Triplonchium-*Arten*

Merkmale: Arten:	*cylindricum*	*minor*	*bulgaricum*	*pileatum*
Körperlänge in mm	♀: 1,2-2,1 ♂: 1,0-1,5	♀: 0,6 ♂: unbekannt	♀: unbekannt ♂: 1,35	♀: 1,19 ♂: 1,23
Kopf	nicht abgesetzt	nicht abgesetzt	nicht abgesetzt	scharf abgesetzt
Lippenpapillen	vorragend	vorragend	vorragend	eingesenkt
Seitenorgan	hinter der Stachelspitze	hinter der Stachelspitze	weit hinter der Stachelspitze	vor der Stachelspitze
Weibliches Rektum	dünn	dünn	dünn (juv.)	dick, stark kutikularisiert
Kaudaler Darmsack	kürzer als ½ der Analbreite	so lang wie die Analbreite	sehr kurz	länger als die Analbreite
Schwanzendkanälchen	fehlt	fehlt	vorhanden	fehlt
Vaginalänge	½ der Körperbreite	1/3 der Körperbreite	—	½ der Körperbreite
Distalende des Spikulums	zugespitzt	—	zugespitzt	fein abgerundet
Proximalfortsatz des Spikulums	kräftig	—	schlank	sehr schlank
Zahl der Präanalorgane	1	—	5	1
Zahl der Postanalpapillen	0	—	2 Paare	0

SUMMARY

A new species of the rare genus Triplonchium, *Cobb, 1920*

A new species of the genus *Triplonchium* is described from sandy soil. *T. pileatum* n. sp. can be distinguished from the other three species of the genus mainly by following characteristics: head sharply offset, cap like, papillae not prominent, amphids in front of distal end of spear, rectum heavily cutinised, well set off, spicula and gubernaculum unique.

SCHRIFTTUM

Andrássy, I. (1958). Erd- und Süsswassernematoden aus Bulgarien. *Acta zool. Hung.* **4**, 1-88.
Cobb, N. A. (1920). One hundred new nemas. *Contr. Sci. Nematol.* **9**, *Baltimore*, 217-343.
Thorne, G. (1939). A monograph of the nematodes of the superfamily Dorylaimoidea. *Capita zool.* **8**, 1-261.

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ON THE IDENTITY OF *DORYLAIMUS ROBUSTUS* DE MAN

BY

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The taxonomic position of *Dorylaimus robustus* De Man, 1876 was uncertain. The original description shows that the species must be placed in the restricted genus *Dorylaimus* (Andrássy, 1959) and may be regarded as synonymous with *D. stagnalis* Dujardin, 1845. De Man's later descriptions of *D. robustus* refer partly to *D. crassus* De Man, 1884, *Aporcelaimus regius* (De Man, 1876) (probably), and *Sectonema* spec. (possibly an undescribed species).

In 1876 De Man described a new nematode species *Dorylaimus robustus* from a single male. The specimen was dissected by him and exists no longer. In 1884 he described another male and a female, and in 1921 he gave a detailed description of a second female specimen.

Skwarra (1922) and Micoletzky (1925) reported specimens doubtfully placed in this species, but apart from this *D. robustus* appears never to have been found again. Its systematic position is uncertain. Thorne & Swanger (1936) and Thorne (1939) suggested that the 1921 female might belong to *Sectonema.* Andrássy (1960) transferred *D. robustus* to *Labronema*; this was based evidently upon the combined characters of double spear guiding ring (male of 1876) and short, blunt female tail (1884 and 1921). However, the descriptions of these females are in some points at variance with that of the holotype. The latter possessed a spear that was about twice the width of the lip region in length, while the ratio "body width at base of oesophagus: diameter of lip region" was 4.7. In the 1884 female this ratio was about 3.5, while the combined length of spear and extension was thrice the width of the lip region, so that the spear proper cannot have been more than 1.5 × as long as the diameter of the lip region. In the 1921 female the spear was distinctly shorter than the width of the lip region. It is therefore certain that the 1921 female was specifically — if not generically — different from the holotype, and highly probable that the 1884 female was too. To establish the identity of *D. robustus,* we must start from the 1876 description only. Careful study of this description convinced the present writer that the status of *D. robustus* can be determined with reasonable certainty.

The original description states: Length of male = 7 mm (corrected value = 6.89 mm); a = 42; b = 5; c = 160. Cuticle very thick. Lips set off, but weakly developed, blunt; papillae not observed. Somatic papillae present in neck region. Spear length equal to one-ninth of oesophagus length. Spicules pointed at both extremities. Forty small midventral preanal supplements in a contiguous series.

The length of the spear would thus be 156 μ, but the illustration 3c (Fig. 1) shows that what De Man called the spear, was actually spear and extension. Real spear length would be about 64 μ. The illustrations 3a and 3b (Fig. 1) show,

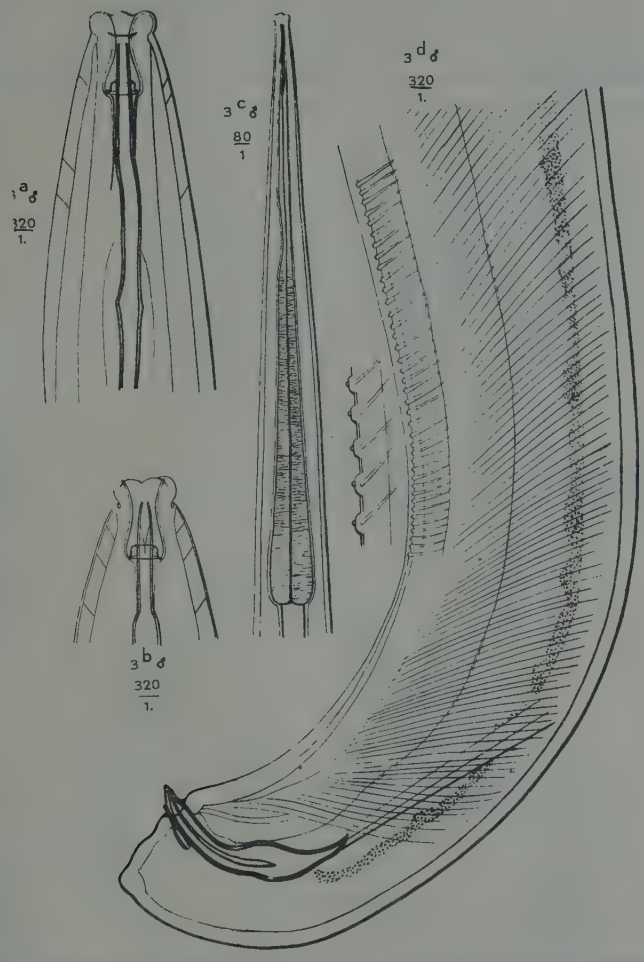

Fig. 1. *Dorylaimus robustus*. From De Man, 1876; by courtesy of Martinus Nijhoff, Publishers. a, b, d × 272; c × 68.

furthermore, that the guiding ring is double; its distance from the head end is equal to 1.2 × the width of the lip region. Two circles of somatic papillae are present at the level of the guiding ring. Body diameter at base of oesophagus equal to 4.7 × width of lip region.

These details indicate that *D. robustus* belongs to the genus *Dorylaimus* in its restricted sense (Andrássy, 1959). Applying Andrássy's key, it keys out either with

D. stagnalis Dujardin, 1845, or with *D. profundis* Cobb, 1904; the dubious point is the presence or absence of longitudinal cuticular striation. In his earlier works De Man seems to have accepted the view of Bütschli (1873) that in all dorylaims such striae were caused by the body musculature. He did not mention them in his 1880 and 1884 descriptions of *D. stagnalis* and it was not until 1907 that he recognized the presence of true longitudinal cuticular striation in that species. Therefore the fact that he did not mention them in his 1876 description of *D. robustus* does not give any indication about their presence or absence in that species.

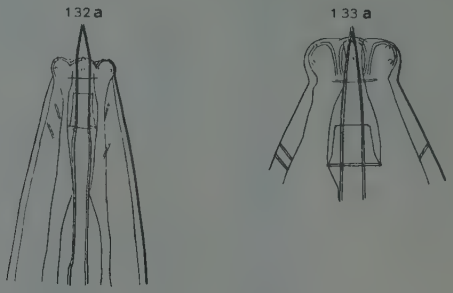

Fig. 2. Two figures from a page of illustrations from De Man, 1884. 132a: *D. stagnalis* ♀; 133a: *D. crassus* ♀

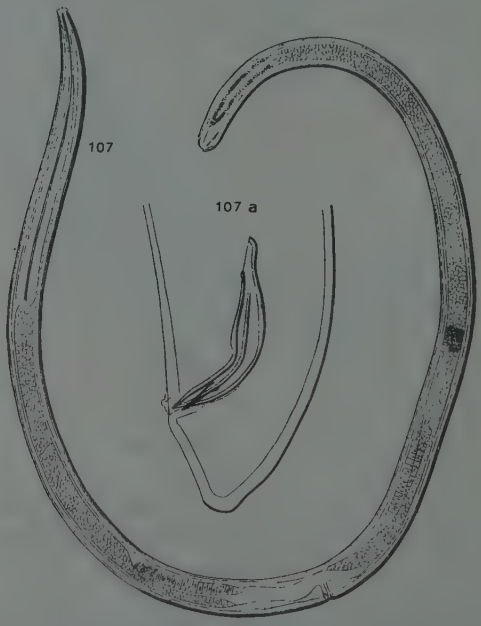

Fig. 3. Specimens described as *D. robustus* by De Man in 1884. From De Man, 1884.

TABLE I

Comparison of data for Dorylaimus robustus *and* D. stagnalis

Character	*D. robustus*	*D. stagnalis* (chiefly from De Man, 1907)	Comment
Length ♂	6.89 mm	5.3 mm	Females of *D. stagnalis* reach 8 mm in size so that it appears quite possible that the male may be as large as 6.89 mm
a	42	40	
b	5	5	
c ♂	160	115	Difference of c presumably due to allometry. Bastian (1865) gave c as 133 in a specimen 5.08 mm in length.
Shape of lip region	offset by constriction	continuous or offset by depression	In 1884 De Man illustrated the lip region of *D. stagnalis* and its close relative *D. crassus* in the same way as *D. robustus* in 1876 (Fig. 2).
Guiding ring	double	double	
Spear length	64 μ	45-65 μ	
Circles of papillae at level of guiding ring	2	2	
Body diameter at base of oesophagus / diameter of lip region	4.7	5-6	
Number of ventromedian supplements	40	45-48	After Thorne & Swanger (1936) 35-45; after Andrássy (1959) 30-50.
Arrangement of supplements	contiguous	contiguous	
Straight distance between extremities of spicules	106 μ	0.1 mm	
Shape of spicules	curved, pointed at both extremities	curved, pointed at both extremities	
Habitat	moist soil, roots of *Phragmites*, near bank of ditch	mainly freshwater, but occasionally found in moist soil	

As far as the writer knows, *D. profundis* has never been found again since its discovery in New Zealand. *D. stagnalis* has a wide distribution; it occurs in the Netherlands too, among other places in the vicinity of Leiden, which is the type locality of *D. robustus*.

The chief characters of *D. robustus* are exposed in Table I, and compared with those of *D. stagnalis*. It is clear that there is no conflict between the description of *D. robustus* as far as it goes, and *D. stagnalis*. In 1876 De Man had not found the latter species, and in 1880/1884 he described only females, not having found males with them. This may explain why he did not recognize the possibility that *D. robustus* and *D. stagnalis* might belong together. Moreover, in 1884 he referred to Bütschli's remark (1873) that midventral preanal supplements seemed to be absent in *D. stagnalis*.

In order to settle the status of *D. robustus* and to prevent its further roaming through the dorylaimid system without any one having seen authentic specimens,

it is herewith proposed to sink *D. robustus* De Man, 1876 into synonymy with *D. stagnalis* Dujardin, 1845. The writer disagrees with Andrássy's placing (1960) of this species in *Labronema*: the lips are not flap-like, the anterior part of the oesophagus is slender, the *Labronema* spicules illustrated by Thorne & Swanger (1936) and Thorne (1939) appear more blunt proximally, the highest number of preanal midventral supplements known in *Labronema* is thirty, and the known *Labronema* species do not surpass 4 mm in length. Moreover, *Labronema* species have not so far been found in the Netherlands, as far as the writer knows.

It remains now to consider the three other descriptions of *D. robustus* by De Man. The specimens reported by Skwarra and Micoletzky are not identifiable.

The 1880 description seems to refer to the same specimen as the 1876 one. Apparently De Man had not found fresh material of *D. robustus* between 1876 and 1879.

The 1884 description of the male refers partly to the holotype of 1876 and partly to a male, 4.2 mm in length, found November 1879 near Leiden. This specimen is still present in De Man's collection and will be redescribed elsewhere. It belongs to *D. crassus* De Man, 1884, a species closely related to *D. stagnalis,* also possessing longitudinal striae. Thus at least one specimen described by De Man under the name *D. robustus* possessed longitudinal striae, although they were not mentioned in the description. This specimen also supports the assumption that the holotype of *D. robustus* belonged to *Dorylaimus* proper.

The female described in 1884 possesses a short blunt tail, which indicates that it does not belong to *Dorylaimus sensu stricto*; it has a typically dorylaimoid oesophagus and therefore is not a *Labronema* either (Fig. 3). It is difficult to establish its systematic position satisfactorily, because De Man gave only a small-scale drawing of the whole specimen. It seems most plausible that it belongs to *Aporcelaimus.* De Man remarked that it was difficult to distinguish the female of *D. robustus* from related forms. Of the *Dorylaimus* species described in 1884 the only one with which the "*robustus*"-female could be confused, is *D.* (now *Aporcelaimus*) *regius.* It is noteworthy that De Man in the "Anmerkung" in the description of *D. robustus* did not give distinguishing characteristics (of the female), contrary to his custom. The legend to the illustration suggests that this female was found together with the *D. crassus* male; this may be the reason why De Man placed it in *D. robustus.* The specimen is no longer present in De Man's collection.

The 1921 female is characterized by spear shape, by the apparent extension ventrally of the pharynx, by the ventral wall of the pharynx being more heavily sclerotized than the dorsal wall, by the oesophagus being enlarged at 28% of its length and by the presence of criss-cross striae on the cuticle. These particulars indicate that it belongs either to *Sectonema,* as Thorne & Swanger (1936) and Thorne (1939) supposed, or to the group of *Aporcelaimus* species with angular spear aperture. As De Man's drawing (Fig. 4) shows that the dorsal wall of the pharynx passes just clear of the spear base, and that on the dorsal side of the

spear there is a distinct angle between the apical and basal portion, the specimen must be placed in *Sectonema*. The taxonomy of this genus is somewhat uncertain, because of *S. ventrale* Thorne, 1930 only the female is known, of *S. rotundicauda* Goodey, 1951 only the male. From the latter species De Man's specimen differs by the more slender shape of the tooth, from the former by a slightly longer tooth (three-fifths of lip region width against one-half or less), by the lower lips and by the shape of the tail. It thus most probably represents an undescribed species, but the writer prefers not to name it on the basis of a specimen which he has not seen and which possibly exists no longer; the less so as he is not sure

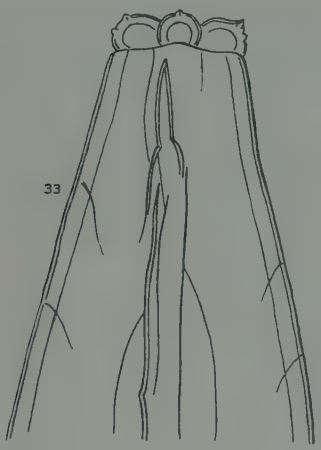

Fig. 4. Specimen described as *D. robustus* by De Man in 1921. From De Man, 1921; by courtesy of Martinus Nijhoff, Publishers. × 680.

whether the differences mentioned are sufficient evidence that the specimen is really specifically different from *S. ventrale*. De Man gave the tooth length as 72 μ, but this obviously is an error, caused by the strongly sclerotized ventral wall of the pharynx (conspicuous at a magnification of about 100 x) and the break at about 55 μ behind the base of the tooth. The real tooth length was rather 17-18 μ.

Summarizing, the following synonymies are herewith proposed:

Dorylaimus robustus De Man, 1876: syn. of *D. stagnalis* Dujardin, 1845.

D. robustus apud De Man, 1880; apud De Man, 1884 (male, partim); *Labronema robustum* (De Man, 1876) Andrássy, 1960: same.

D. robustus apud De Man, 1884 (male, partim): syn. of *D. crassus* De Man, 1884.

D. robustus apud De Man, 1884 (female): probably *Aporcelaimus* spec.

D. robustus apud De Man, 1921: *Sectonema* spec., probably an undescribed species.

ZUSAMMENFASSUNG

Über die Identität von Dorylaimus robustus *de Man*

Die systematische Stellung von *Dorylaimus robustus* De Man, 1876 war bis jetzt ungewiss. Die Originalbeschreibung zeigt jedoch, dass die Art zu *Dorylaimus sensu stricto* (Andrássy, 1959) gehört und als Synonym von *D. stagnalis* Dujardin, 1845 angesehen werden darf. De Man's spätere Beschreibungen von *D. robustus* beziehen sich teilweise auf *D. crassus* De Man, 1884, *Aporcelaimus* spec. (nicht ganz sicher) und eine vermutlich unbeschriebene *Sectonema*-Art.

LITERATURE CITED

Andrássy, I. (1959). Taxonomische Uebersicht der Dorylaimen (Nematoda) I. *Acta zool. Acad. Sci. Hung.* **5**, 191-240.

—— (1960). Taxonomische Uebersicht der Dorylaimen (Nematoda) II. *Ibid.* **6**, 1-28.

Bastian, H. Ch. (1865). Monograph on the Anguillulidae, or Free Nematoids, Marine, Land and Freshwater, with Descriptions of 100 New Species. *Trans. Linn. Soc.* **25**, 73-184.

Bütschli, O. (1873). Beiträge zur Kenntnis der freilebenden Nematoden. *Nova Acta Leop. Carol.* **36**, 1-144.

Goodey, T. (1951). *Soil and freshwater nematodes.* London, Methuen, 390 pp.

De Man, J. G. (1876). Onderzoekingen over vrij in de aarde levende Nematoden. *Tijdschr. Ned. dierk. Ver.* **2**, 78-196.

—— (1880). Die einheimischen, frei in der reinen Erde und im süssen Wasser lebenden Nematoden. *Ibid.* **5**, 1-104.

—— (1884). *Die frei in der reinen Erde und im süssen Wasser lebenden Nematoden der niederländischen Fauna.* Leiden, Brill, 206 pp.

—— (1907). Contribution à la connaissance des nématodes libres de la Seine et des environs de Paris. *Ann. Biol. Lacust.* **2**, 9-29.

—— (1921). Nouvelles recherches sur les nématodes libres terricoles. *Cap. zool.* **1**, 1-62.

Micoletzky, H. (1925). Die freilebenden Süsswasser- und Moornematoden Dänemarks. *K. danske vidensk. Selsk.* **8**, 57-310.

Skwarra, E. (1922). Freilebende Nematoden Ostpreussens. *Schr. phys.-ökon. Ges. Königsb.* **63**, 107-112.

Thorne, G. (1939). A monograph of the nematodes of the superfamily Dorylaimoidea. *Cap. zool.* **8**, 1-261.

Thorne, G. & Swanger, H. H. (1936). A monograph of the nematode genera *Dorylaimus* Dujardin, *Aporcelaimus* n.g., *Dorylaimoides* n.g. and *Pungentus* n.g. *Ibid.* **6**, 1-223.

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THE NATURE OF RESISTANCE IN CHRYSANTHEMUM VARIETIES TO *APHELENCHOIDES RITZEMABOSI*

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Leaves of resistant varieties of chrysanthemum brown quickly when infested with *A. ritzemabosi*. In such leaves there is no eelworm multiplication and so the infestation is isolated and does not spread. There is no difference in the rate of browning of aerated extracts of leaves of resistant and susceptible varieties and no differences in polyphenolic content or polyphenol oxidase concentration. Infested leaves of resistant and susceptible varieties brown at different rates because in leaves of resistant varieties the gravid females move about in the leaf, piercing hundreds of cells which subsequently turn brown. In susceptible varieties the females do not move, they pierce few cells and lay many eggs. This difference in behaviour is attributed to the absence of a nutritional factor in leaves of resistant varieties.

Data from field experiments suggest that varietal resistance of chrysanthemums to *Aphelenchoides ritzemabosi* (Schwartz) is caused by a hypersensitive browning reaction of the leaf to eelworm infestation (Hesling & Wallace, 1960). This association between rate of leaf browning and resistance to eelworm poses two questions. First, why do infested leaves of some varieties brown quicker than others? Second, what affect has browning on the eelworm? This paper attempts to elucidate the nature of resistance by considering these questions.

MATERIALS AND METHODS

Techniques for the culture of individual chrysanthemum leaves, inoculation of leaves with eelworms, staining of infested leaves, chromatography of leaf polyphenols and preparation of polyphenol-oxidase have been described in previous papers (Wallace, 1961).

The relative quantities of the major polyphenols were estimated by taking eight similar leaves from each of five plants of three resistant and three susceptible varieties. Seventy-five leaf discs, 1 cm in diameter were taken from each sample of eight leaves. Twenty-five discs were weighed and then heated to dryness at 105°C and weighed again. Quantities of polyphenols could then be expressed in terms of leaf area, fresh weight and dry weight. The remaining fifty leaf discs were macerated in an Atomix 100 homogeniser for 1 minute, filtered, the filtrate concentrated to a volume of 5 ml by rotary evaporation and centrifuged. 0.1 ml of each of the five samples in each of the six varieties was then spotted on to Whatman's no. 1 chromatography paper and run one way in 6 per cent acetic acid. After drying, the major spots were cut out under U.V. and the polyphenols

eluted from the paper by immersion in 80 per cent methyl alcohol for 24 hours. The absorption of each sample was determined in a Unicam spectrophotometer at a wavelength of 330 mμ for chlorogenic acid and isochlorogenic acid and 350 mμ for the glycoside of luteolin. These wavelengths correspond to the absorption maxima for each compound.

To determine relative quantities of polyphenol oxidase, samples of leaves were obtained as just described but with three samples per variety. The enzyme solu-

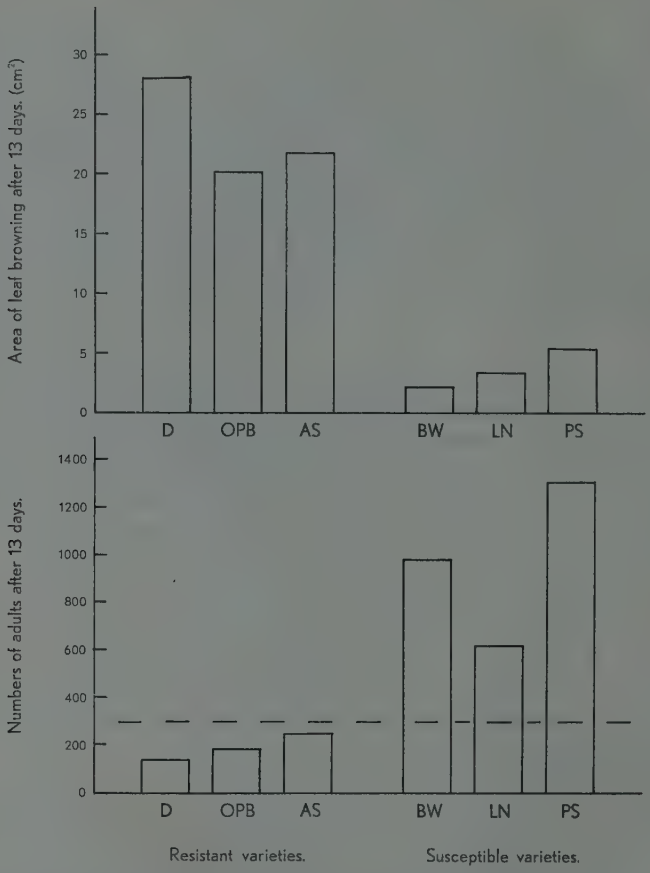

Fig. 1. Rate of browning and eelworm multiplication in leaves of three resistant varieties, Delightful (D), Orange Peach Blossom (OPB), Amy Shoesmith (AS) and three susceptible varieties, Bill Walters (BW), Lilac Nymph (LN) and Pennine Snow (PS). Each histogram is the mean of five replicates and the dotted line in the bottom graph shows the level of the original eelworm inoculum.

tions, obtained by maceration of leaves in phosphate buffer (pH7) and dialysis against distilled water for 24 hours, were stored at 0°C. Four polyphenol oxidase concentrations from variety Orange Peach Blossom were prepared using 0.2, 0.4,

0.5 and 1.0 ml of enzyme solution in 25 ml of DL-b-(3, 4 Dihydroxy-phenyl) Alanine (Dopa) solution (50 mg Dopa per 100 ml phosphate buffer pH7). The change in colour of the four solutions was measured at intervals of 1 minute for 10 minutes in a Hilger and Watts Spekker absorptiometer. The graphs of absorption with enzyme concentration and with time were linear indicating the validity of using absorption in a Dopa-enzyme mixture as a criterion of enzyme concentration. To compare the polyphenol oxidase concentrations in the three samples in each of the six varieties, 0.5 ml of each enzyme preparation was mixed with 25 ml of Dopa solution and the absorption measured after 5 minutes.

The technique for estimating the rate of browning of each of the six varieties in aerated phosphate buffer solutions has been described previously (Wallace, 1961).

RESULTS

Rate of leaf browning and eelworm multiplication

Five leaves from each of the resistant varieties Orange Peach Blossom (OPB), Amy Shoesmith (AS), and Delightful (D) and susceptible varieties Lilac Nymph (LN), Bill Walters (BW) and Pennine Snow (PS) were suspended in glass tanks with the petioles in water. About 300 eelworms were placed in a drop of water on the underside of each leaf and covered with a polythene strip. After 13 days the brown area on each leaf was measured and the leaf immersed in a shallow layer of water for 2 days. The adults that emerged from the leaves were counted. The results (Fig. 1) showed that the resistant varieties had a higher rate of browning and no eelworm multiplication whereas the susceptible varieties had a low rate of browning with eelworm multiplication. In resistant varieties the first symptoms appeared about 4 to 8 hours after infestation and after 4 days nearly half the leaf was brown. Fig. 5 shows leaves of the six varieties after 4 days infestation with 300 eelworms. Amy Shoesmith and Orange Peach Blossom leaves have brown sectors on one side of the main vein and Delightful is almost wholly discoloured. The three susceptible varieties show no symptoms. These results confirm the hypothesis that resistance is a form of hypersensitivity.

Field observations

Cuttings of resistant and susceptible varieties artificially infested in March 1960 were examined in September for leaf symptoms and the dead leaves at the base of the plant were counted. Eight plants in each of the susceptible varieties Pennine Snow and Bill Walters had a mean of 21.5 and 11.8 dead leaves per plant respectively whereas eight plants of the resistant variety Orange Peach Blossom had 2.4. The dead leaves were immersed in water and the eelworms that emerged were counted after 2 days. Pennine Snow and Bill Walters produced about 730 and 490 eelworms per leaf compared with 8 per leaf for Orange Peach Blossom.

Development of leaf symptoms in resistant and susceptible varieties is different.

In susceptible varieties the brown discolouration spreads across the leaf to give distinct brown areas between the main veins. In resistant varieties, however, the interveinal symptoms appear only in the early stages, thereafter the infested tissues become black and brittle during dry weather and are often surrounded by a yellow band which is not confined by the veins.

A single leaf on each of eight plants in varieties Pennine Snow, Bill Walters and Orange Peach Blossom was infested in early August 1960 with about 200

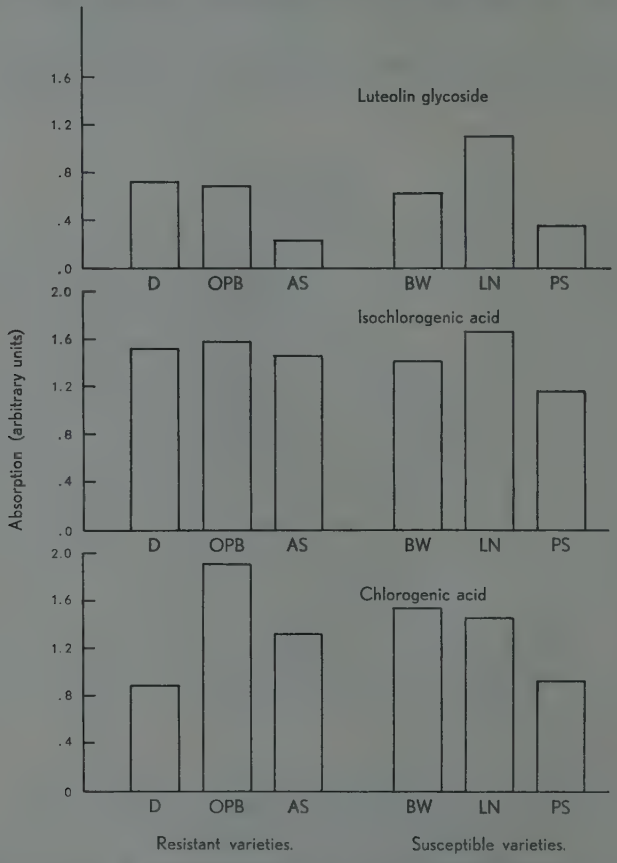

Fig. 2. Relative quantities of luteolin glycoside, isochlorogenic acid and chlorogenic acid from equal leaf areas in three resistant and three susceptible varieties of chrysanthemum. Each histogram is the mean of five replicates.

eelworms by the polythene strip method. Two months after infestation the susceptible varieties (PS and BW) had leaves with symptoms above the original infested leaf. No upward spread of symptoms was apparent in the resistant variety (OPB).

These observations suggest that infested leaves on resistant plants brown so quickly that multiplication of eelworms is inhibited and consequently there is little spread up the plant with little indication of dead leaves.

Rate of browning in aerated leaf extracts

Fifty leaf discs from the three resistant and three susceptible varieties were macerated in phosphate buffer, centrifuged and the supernatant fluid poured into petri dishes. The leaf extracts were sampled ½, 1 and 1½ hours after homogenisation and spotted on to chromatography paper. The chromatograms were run in 6 per cent acetic acid, dried and then examined under U.V. The chlorogenic acid and isochlorogenic acid spots in all varieties decreased with time and had

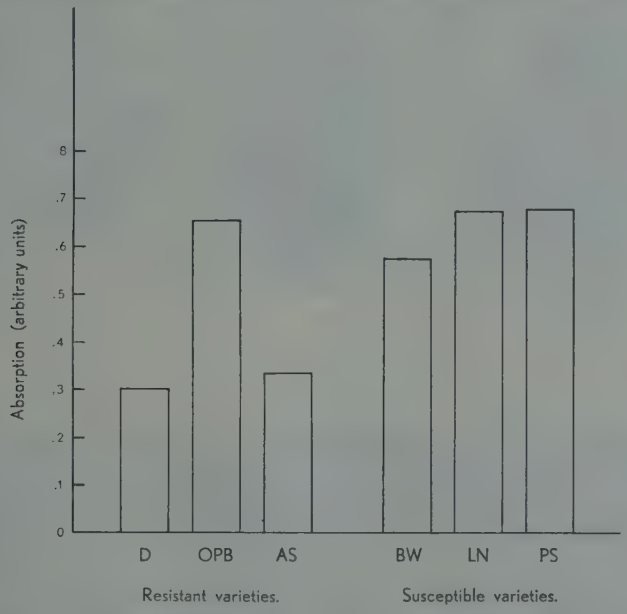

Fig. 3. Relative concentrations of polyphenol oxidase from equal leaf areas in three resistant and three susceptible varieties of chrysanthemum. Each histogram is the mean of three replicates.

disappeared after 1½ hours. No differences were detected between the resistant and susceptible varieties. Wallace (1961) has shown that isochlorogenic acid and chlorogenic acid are probably the chief substrates for browning. Thus, there is no apparent difference in the rate of enzymic oxidation between resistant and susceptible varieties.

A comparative estimation of the major polyphenols in terms of dry weight, fresh weight and leaf area showed no differences which could be correlated with resistance (Fig. 2). Similarly, estimations of polyphenol oxidase activity (Fig. 3) gave no indication of such a relationship. The hypothesis that leaves of resistant

varieties brown quickly because they contain more polyphenol substrate or polyphenol oxidase is, therefore, not valid.

Behaviour of females in leaves of resistant and susceptible varieties

Five leaves in three resistant (OPB, AS, D) and three susceptible (PS, LN, BW) varieties were each infested with five gravid females. After 6 days the leaves were stained in acid-fuchsin-lactophenol and the adults, eggs and larvae counted in each leaf. The results expressed as eggs and larvae per female are given in Fig. 4. Two to four eggs per female were laid in the resistant varieties compared with sixteen to twenty-three in the susceptible ones.

Examination of the stained leaves showed that in resistant varieties the female was surrounded by hundreds of brown cells (Fig. 6) whereas in the susceptible varieties there was little indication of browning (Fig. 7). Furthermore, in the resistant varieties the female moved extensively through the leaf often leaving

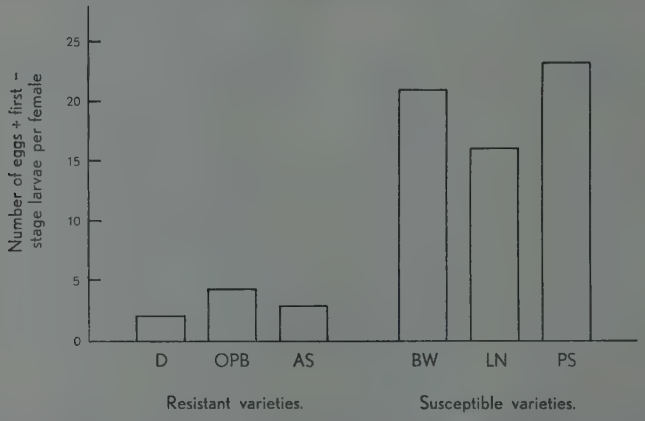

Fig. 4. Eelworm multiplication in resistant and susceptible varieties of chrysanthemum using an inoculum of five gravid females per leaf. Each histogram is the mean of five replicates.

brown tracks (Fig. 8) whereas in susceptible varieties the female and its eggs were in a compact group suggesting that little movement occurred within the leaf.

The observations suggest that differences in rate of browning are related to the number of cells which the eelworm pierces during feeding. In resistant varieties the female pierces hundreds of cells, lays few eggs and moves about a great deal in the leaf. In susceptible varieties few cells are pierced, many eggs are laid and the female moves very little. It is suggested that this difference in behaviour is caused by the lack of some unknown nutritional factor in leaves of resistant varieties which causes more cells to be pierced and which reduces the number of eggs laid. This hypothesis receives support from earlier work (Wallace, 1960) which showed that egg laying in an infested leaf only occurred in the green tissues where eelworms could feed. Thus rate of browning, feeding and reproduction are interdependent factors which form the basis of resistance.

Fig. 5. Browning caused by eelworms after 4 days in chrysanthemum leaves. The resistant varieties (top row, left to right) Orange Peach Blossom, Amy Shoesmith and Delightful show symptoms whereas there is no discolouration in the susceptible varieties (bottom row, left to right) Lilac Nymph, Pennine Snow and Bill Walters.

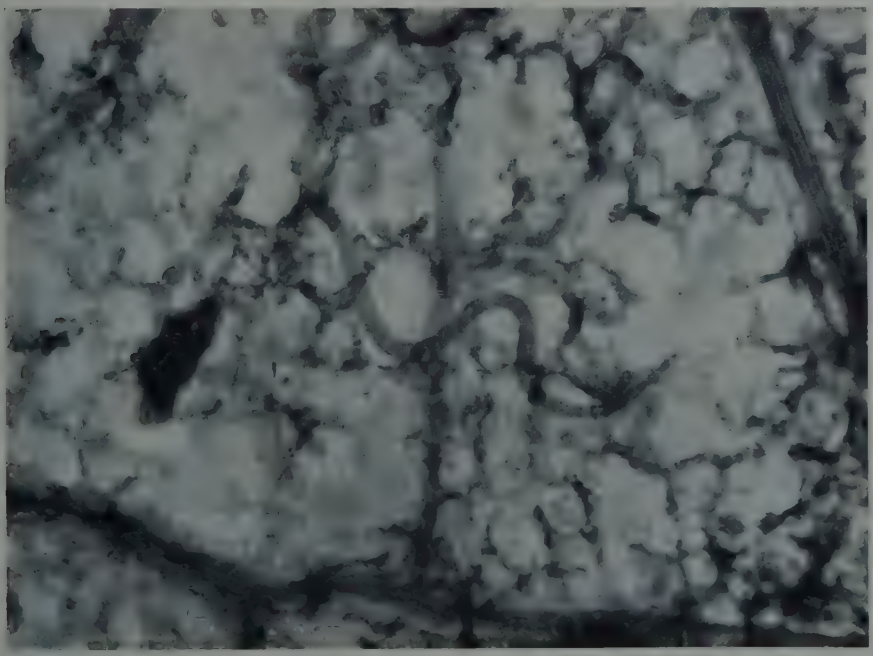

Fig. 6. A female of *A. ritzemabosi* in a leaf of a resistant variety of chrysanthemum, Amy Shoesmith. The eelworm is surrounded by brown dead cells and no eggs are present. The female is about 900 μ long.

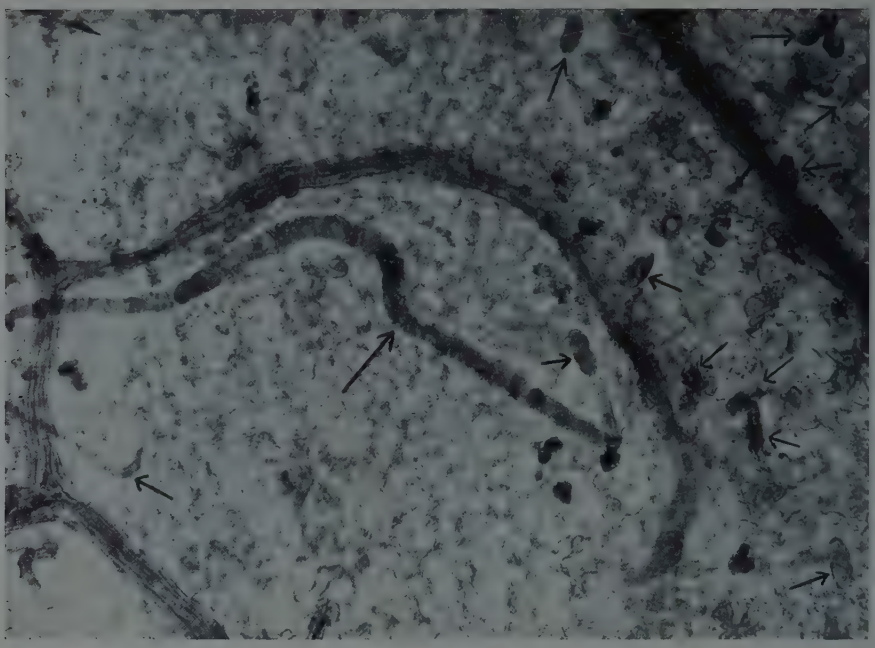

Fig. 7. A female *A. ritzemabosi* (large arrow) in a leaf of a susceptible variety, Pennine Snow. There are few dead brown cells and of the twenty-three eggs close to the female, ten are visible in this photograph (small arrows) plus one newly hatched larva on the left of the picture. The female is about 900 μ long.

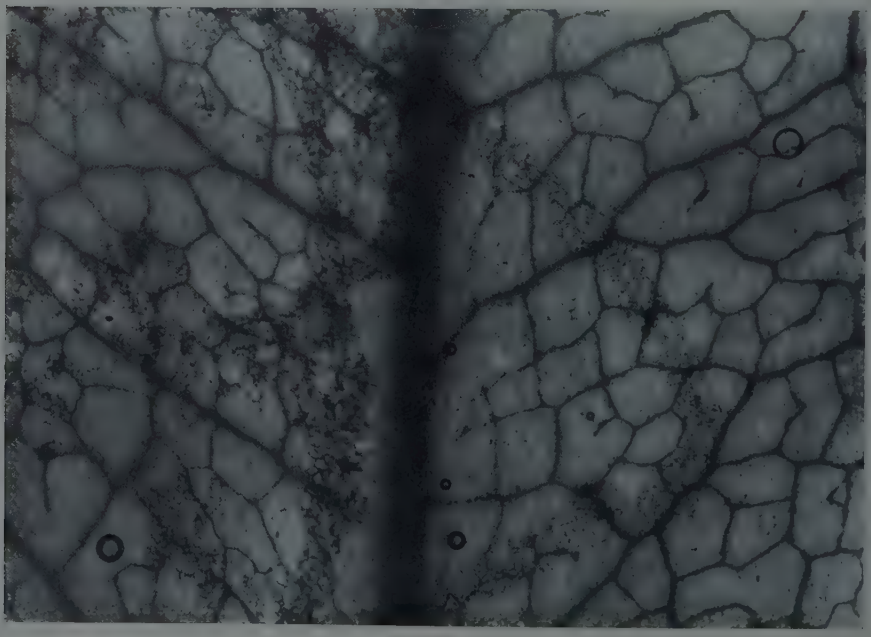

Fig. 8. Tracks of brown dead cells left by migrating females of *A. ritzemabosi* in a leaf of resistant variety Amy Shoesmith. The tracks are about 500 μ across.

The influence of polyphenols and quinic acid on eelworms

During browning chlorogenic acid and isochlorogenic acid are hydrolysed to caffeic acid which is then oxidised enzymically to quinic acid followed by polymerisation to the brown end products. It is possible that in the infested leaf these chemicals may affect reproduction. To study this, 0.01 M solutions of quinic acid and chlorogenic acid, a saturated solution of caffeic acid, a solution obtained

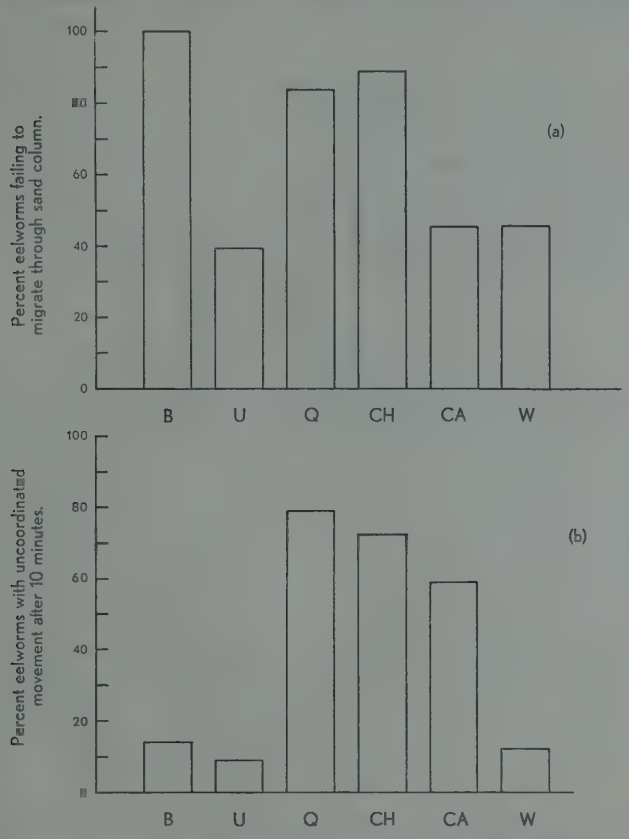

Fig. 9. The influence of brown leaf extract (B), Urea (U), Quinic acid (Q), Chlorogenic acid (CH), Caffeic acid (CA), and water on migration and coordinated movement of *A. ritzemabosi*. Each histogram is the mean of five replicates.

by macerating ten browned chrysanthemum leaves in 70 ml of water and distilled water were prepared. A 0.01 M urea solution was also used as a further control to test any osmotic pressure affects. About 100 eelworms were placed in the solutions in solid watchglasses. The eelworms remained active in all the solutions, but in quinic acid, chlorogenic acid and caffeic acid the usual undulatory movements were replaced by twisting and coiling after 10 minutes (Fig. 9b). Ability to move

through sand saturated by these six chemicals was also studied by a method described by Wallace (1958). About 200 eelworms were placed on the top of a vertical saturated column of sand (250-500 μ) 2.5 cm long and 5 mm diameter and the percentage which failed to migrate through the column was estimated. The results (Fig. 9a) showed that movement was inhibited in the quinic acid and chlorogenic acid; the eelworms left in the sand showed uncoordinated movement. The brown solution also inhibited movement but this was attributed to lack of

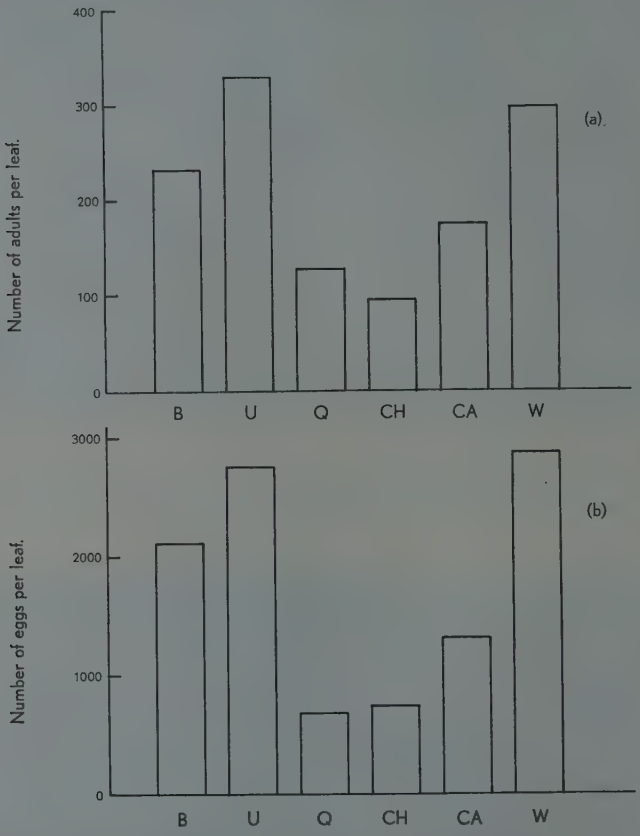

Fig. 10. The influence of brown leaf extract (B), Urea (U), Quinic acid (Q), Chlorogenic acid (CH), Caffeic acid (CA) and water on (a) the eelworm invasion of leaves and (b) numbers of eggs laid in the leaf.

aeration because eelworms in thin layers of this solution remain active for days. The similarity between the urea and water treatments indicated that inhibition of movement was not caused by an osmotic affect.

Chrysanthemum eelworms were placed in the six solutions for 24 hours and from each solution five lots of about 300 were taken to infest leaves of variety

Pennine Snow. After 5 days the leaves were stained and then macerated in an Atomix 100 homogeniser for ½ min. which broke up the leaf but left the eelworms stained and intact. Aliquots were taken from the suspension and the adults and eggs counted (Fig. 10). The number of adults invading the leaf and the number of eggs in the leaves were reduced by pretreatment with quinic acid, chlorogenic acid and caffeic acid. The relationship between numbers of eggs laid and numbers of adults invading the leaf is very close, consequently, whereas these results show that invasion may be inhibited, there is no evidence that egg laying is affected.

These observations show that chlorogenic acid, quinic acid and caffeic acid at a concentration of 0.01 M. affect the movements of chrysanthemum eelworm. Observations, however, have failed to show any such inhibition in infested leaves; in fact most active migration occurs in the rapidly browning leaves of resistant varieties (Fig. 8). There is also no evidence that egg laying is inhibited by these chemicals. Thus, it is doubtful whether polyphenols or their oxidation products affect eelworms at the concentration which occur in the browning leaf.

DISCUSSION

Resistance in some varieties of chrysanthemums to *A. ritzemabosi* is not related to differences in phenolic content nor polyphenol oxidase concentration in the leaves but probably to the lack of some nutritional factor. The identity of this factor is unknown and it is difficult to envisage any progress along these lines until *A. ritzemabosi* can be cultured on artificial media of known chemical constitution. Although the enzymic oxidation of polyphenols during browning of infested leaves does not appear to be the cause of resistance, it is probable that rapidly browning tissues expose larvae and eggs to desiccation. Wallace (1960) showed that the length of life-cycle of *A. ritzemabosi* is about 10 to 13 days and that leaf sectors brown in only 3 to 4 days, consequently, the few larvae which do develop in leaves of resistant varieties probably never become adults. The lack of a hypothetical nutritional factor and the physical conditions in the browning leaf are, therefore, the primary causes of resistance in chrysanthemums to *A. ritzemabosi.*

ZUSAMMENFASSUNG

Die Natur der Resistenz gegenüber Aphelenchoides ritzemabosi *bei Chrysanthemumsorten*

Blätter resistenter Sorten von Chrysanthemum werden schnell braun, wenn sie von *Aphelenchoides ritzemabosi* befallen sind. In solchen Blättern tritt keine Älchenvermehrung ein. Der Befall ist isoliert und breitet sich nicht aus. Beobachtungen an künstlich infizierten Pflanzen im Feld stützen die Hypothese einer Überempfindlichkeit. Es gibt keinen Unterschied in der Höhe der Braunfärbung belüfteter Blattextrakte resistenter und anfälliger Sorten und keine Unterschiede im Polyphenolgehalt oder der Polyphenoloxydasemenge. Befallene Blätter resistenter und anfälliger Sorten werden in verschiedenem Grade braun, weil in den Blättern resistenter Sorten die eiertragenden Weibchen sich im Blatt bewegen und hunderte von Zellen anbohren, die anschliessend braun werden. Bei anfälligen Sorten bewegen sich die Weibchen nicht, sie bohren einige Zellen an und legen viele Eier. Dieser Unterschied im Verhalten wird dem Fehlen eines Nahrungsfaktors in

Blättern resistenter Sorten zugeschrieben. Obwohl 0,01 M Chlorogensäure und Quininsäure und eine gesättigte Lösung van Kaffeesäure die Älchenbewegung und das Eindringen verhindern, wurden solche Wirkungen weder in befallenen Blatt beobachtet noch ist die Eiablage beeinflusst. Das Fehlen eines hypothetischen Nahrungsfaktors und die physikalischen Bedingungen in dem sich braun verfärbenden Blatt, das die Larven der Trockenheit aussetzt, sind die primären Ursachen der Resistenz bei Chrysanthemum gegenüber *A. ritzemabosi.*

REFERENCES

HESLING, J. J. & WALLACE, H. R. (1960). Susceptibility of varieties of chrysanthemum to infestation by *Aphelenchoides ritzemabosi* (Schwartz). *Nematologica* **5**, 297-302.

WALLACE, H. R. (1958). Movement of eelworms. I. The influence of pore size and moisture content of the soil on the migration of larvae of the beet eelworm, *Heterodera schachtii* Schmidt. *Ann. appl. Biol.* **46**, 74-85.

—— (1960). Observations on the behaviour of *Aphelenchoides ritzemabosi* in chrysanthemum leaves. *Nematologica* **5**, 315-321.

—— (1961). Browning of chrysanthemum leaves infested with *Aphelenchoides ritzemabosi. Nematologica* **6**, 7-16.

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GYMNOTYLENCHUS ZEAE, N. G., N. SP. (NEMATODA: NEOTYLENCHIDAE), A ROOT ASSOCIATE OF *ZEA MAYS* L. (SWEET CORN) IN ALIGARH, NORTH INDIA

BY

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A new nematode belonging to the family Neotylenchidae is commonly found infesting corn roots at Aligarh (U.P.), India. As it shows certain peculiar morphological characters, especially the absence of a bursa and gubernaculum in male, a new genus and species, *Gymnotylenchus zeae* n. g., n. sp., is proposed and described.

Few additions have been made to the Neotylenchidae since the work of Thorne (1941). Although many species of this family are associated with plant tissues and some, such as *Neotylenchus abulbosus* Steiner, 1931, and *Hexatylus viviparus* Goodey, 1926, have been found in great numbers, their significance as plant parasites is rather doubtful. The present study, however, deals with a new neotylenchid which appears to be an important parasite of corn roots.

In October, 1958, the writer found a heavy infestation of the roots of sweet corn (*Zea mays* L.) in Aligarh, North India, with a nematode apparently belonging to Neotylenchidae. The same nematode was again collected in large numbers from the roots of corn in various fields from the same locality in 1960. The eelworm occurs in all stages of development within the roots. It is, perhaps, specific towards corn because several thousand soil and root samples from various other plants examined by the writer during the last five years failed to reveal its presence.

Morphologically, there are certain remarkable features exhibited by this nematode which give it a unique position among the Neotylenchidae. These include the octagonal labial frame-work, oesophageal glands lying in a diverticulum overlapping the anterior end of the intestine and the absence of a bursa and gubernaculum in male. It is, therefore, proposed to erect a new genus, *Gymnotylenchus*, under Neotylenchinae of the Neotylenchidae, and the eelworm is hereby named *Gymnotylenchus zeae*, n. g., n. sp.

Gymnotylenchus, n. g.

Generic diagnosis. — Neotylenchinae: Body slender, less than 1 mm long, bearing fine transverse striae. Head low, flattened. Winged lateral fields present. Labial frame-work in 8 sectors, lightly sclerotized. Buccal spear well developed,

about 10 μ long, with three rounded basal knobs. Orifice of dorsal oesophageal gland close to spear base. Corpus a cylindrical tube, with a valveless basal swelling. Isthmus not forming basal oesophageal bulb, joined directly with the intestine. Oesophago-intestinal junction indistinct. Oesophageal glands lying in a diverticulum overlapping the anterior end of the intestine. Vulva slit-like, located in the posterior half of the body at less than 90 per cent of the body length from the anterior end. Ovary single, outstretched anteriorly. Testis single, outstretched. Spicules short, weak, almost straight, strongly cephalated. Bursa and gubernaculum absent. Tails of both sexes elongate-cylindroid, regularly tapering, ending in a bluntly rounded terminus. *Type species.* — *Gymnotylenchus zeae,* n. g., n. sp.

Gymnotylenchus zeae, n. g., n. sp.

(Fig. 1, A-I)

Measurements. — 20 females: L. = 0.48-0.63 mm.; a = 34-43; b = 5.5-8.5; c = 10-15; V = 80-84%; spear = 9-11 μ.

10 males: L. = 0.5-0.61 mm.; a = 35-41; b = 6-9; c = 15-18; T = 37-83%; spear = 8-9.5 μ; spicules = 11-14 μ.

Larvae. — Group I, 4 larvae: L. = 0.337-0.35 mm.; a = 26.3-31; b = 5.7-6.1; c = 9.5-12; spear = 8 μ.

Group II, 2 larvae: L. = 0.42-0.43 mm.; a = 35-35.3; b = 5.9-6; c = 12.2-12.9; spear = 8.5-9 μ.

Group III, 2 larvae: L. = 0.456-0.46 mm.; a = 38-38.3; b = 5.8-5.9; c = 12.4-12.5; spear = 9 μ.

Group IV, 3 pre-adult females: L. = 0.48-0.486 mm.; a = 39-40.5; b = 5.6-6; c = 12-13.1; V = 81-81.8%; spear = 9-10 μ.

Female (Holotype). — L. = 0.54 mm.; a = 36; b = 7.5; c = 12.8; V = [48] 81.5%.

Body slender, tapering considerably at either ends, assuming a straight position when the worm is killed by gradual heating. Cuticle marked by fine transverse striae measuring 0.9 μ apart on mid-body. Lateral fields narrow, with faintly marked incisures possibly six in number. Deirids and phasmids not seen. Lip region low, flattened, bearing four transverse striae, set off from body contour, showing 8 almost equal sectors in *en face* view (Fig. 1, B). Labial papillae and amphids not seen. Cephalic frame-work octa-radiate, lightly sclerotized. Outer margins of labial frame-work extending posteriorly three body annules from basal plate. Buccal spear of moderate strength, 10 μ in length, with 5 μ long anterior tapering tip. Basal knobs of spear rounded, 2.7 μ across, attached to the labial frame-work through powerful protrudor muscles. Outlet of dorsal oesophageal gland 2 μ behind spear base.

Corpus a cylindrical tube, 4 μ wide, with a valveless basal swelling containing spherical refractive globules resembling those present in the intestine. Muscular

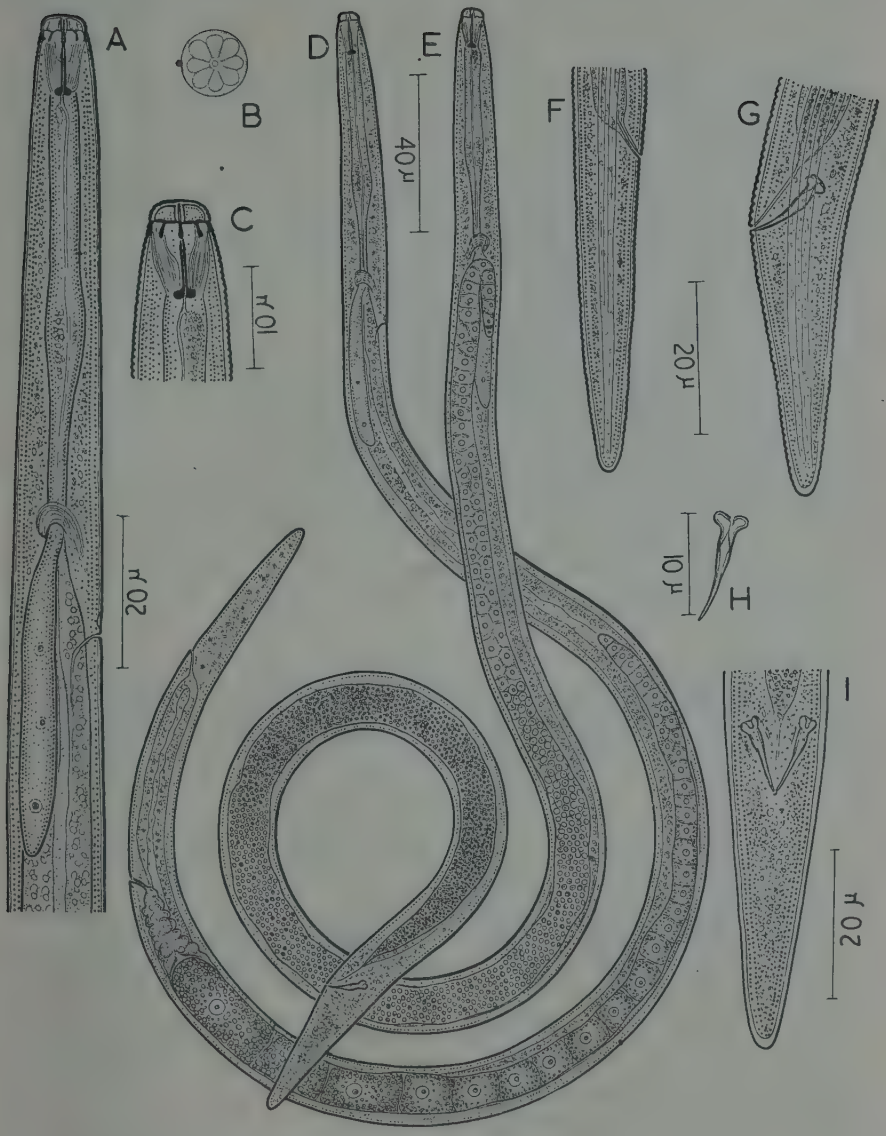

Fig. 1. *Gymnotylenchus zeae:* A. Oesophageal region of female; B. *En face* view; C. Anterior end of male; D. Female; E. Male; F. Female tail in lateral view; G. Male tail in lateral view; H. Spicule; I. Male tail in ventral view.

median oesophageal bulb with its valvular apparatus absent. Isthmus narrower than corpus, joined imperceptibly to intestine, not forming a basal oesophageal bulb. Cardia absent. Oesophageal glands amalgamated, lying in an elongate diverticulum, extending back over anterior end of intestine (Fig. 1, A). Nerve ring enveloping isthmus just anterior to oesophago-intestinal junction, one body width anterior to level of excretory pore. Hemizonid distinct, three body annules long, located two body annules anterior to excretory pore.

Vulva a depressed transverse slit, 5 μ wide, located at 81.5 per cent of the body length. Vagina with thick walls, one-third vulvar-body-width long, leading inwards and forwards. Uterus highly muscular, with folded walls. A distinct spermatheca absent; minute sperms stored in the uterus itself. Ovary single, prodelphic, with oöcytes arranged in single file except for a few in region of multiplication. In certain paratypes, ovary reaching up to oesophageal region. Post-uterine branch absent. Vulva-anus distance greater than tail length.

Intestine with distinct lumen throughout; its cells packed with spherical food granules. Rectum short, about half as long as maximum body width. Anus distinct. Tail about three-and-a-half times anal-body-diameter in length, cylindroid, slightly but regularly tapering to an unstriated, conoid-obtuse terminus.

Male (Allotype). — L. = 0.55 mm.; a = 36; b = 8.3; c = 15.7; T = 82.5%.

Body similar to that of female except for being almost cylindrical up to tail in the posterior region. Buccal spear structurally similar to that of female, 9.5 μ long. Deirids and phasmids not observed. Oesophagus and its glands as described for female.

Testis single, outstretched with reflexed tip, reaching close to oesophago-intestinal junction. Spermatocytes first arranged in single, then in double rows. Vas deferens packed with numerous minute spermatozoa. Spicules paired, slender, almost straight, sharply cephalated, 12 μ in length. Proximal ends of spicules widely separated from each other. Bursa and gubernaculum absent. Tail cylindroid, regularly tapering to a broadly rounded, unstriated terminus.

Larvae. — Body similar to that of female, assuming a straight position on death. Head, buccal spear and oesophagus as described for female. Tail elongate-cylindroid, ending in a rounded terminus.

Holotype. — Female collected on 3rd November, 1958; tube no. PN/N 1-001, deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U.P.), India.

Allotype. — Male; tube no. PN/N/1-002; other data same as for holotype.

Paratypes. — Hundreds of females and males; other data same as for holotype.

Type host. — *Zea mays* L. (Sweet corn).

Type locality. — Aligarh (U.P.), India.

Hosts, habit and geographical distribution. — Specimens of this species have been collected in large numbers from roots of sweet corn (*Zea mays* L.) and *Sorghum vulgare* Pers., in Aligarh (U.P.), India. The worms have been found within the root tissues where they lay eggs and form colonies. Only few specimens

were recovered from soil associated with plant roots. When isolated in water they make very swift wriggling movements.

DISCUSSION

By the character set out above, *Gymnotylenchus*, n. g., apparently belongs to Neotylenchinae of the family Neotylenchidae Thorne, 1949. From all the existing genera of Neotylenchinae this genus is distinguished by the absence of the bursa and gubernaculum in the male. It can be distinguished from *Neotylenchus* Steiner, 1931, and *Hexatylus* Goodey, 1926, by the oesophageal glands lying in a diverticulum overlapping the anterior end of the intestine. Furthermore, the structure of the buccal spear distinguishes this genus from *Hexatylus*. In the structure of the oesophagus, *Gymnotylenchus* comes close to *Deladenus* Thorne, 1941, but can easily be distinguished from it by the presence of cylindroid tails in both the sexes, the position of the vulva at less than 90 per cent of the body length from the anterior end, and the absence of a bursa and gubernaculum in male.

Acknowledgments. The author thanks Dr. M. A. Basir under whose guidance this work was done. He is also indebted to Dr. J. B. Goodey, Rothamsted Experimental Station, Harpenden, England, for valuable advice.

ZUSAMMENFASSUNG

Gymnotylenchus zeae *n. g., n. sp. (Nematoda: Neotylenchidae), ein Wurzelbewohner von* Zea mays *L. in Aligarh, Nord Indien*

Gymnotylenchus zeae n. g., n. sp. der in Maiswurzeln in Aligarh, Nordindien gefunden wurde, wird beschrieben und abgebildet. Die neue Gattung *Gymnotylenchus* gehört zur Unterfamilie der Neotylenchinae und unterscheidet sich von allen beschriebenen Gattungen dieser Unterfamilie durch die Oesophagusdrüsen die in der Leibeshöhle frei liegen, durch die zylindrische Form des Schwanzes in beiden Geschlechtern, und durch das Fehlen von Bursa und Gubernakulum bei dem Männchen.

REFERENCES

GOODEY, T. (1926). *Hexatylus viviparus* gen. et sp. nov., a nematode found in a diseased potato tuber.— *J. Helm.* **4**, 27-30.

STEINER, G. (1931). *Neotylenchus abulbosus* n. g., n. sp. (Tylenchidae, Nematoda). The causal agent of a new nematosis of various crop plants. *J. Wash. Acad. Sci.* **21**, 536-538.

THORNE, G. (1941). Some nematodes of the family Tylenchidae which do not possess a valvular median esophageal bulb. *Gr. Basin Nat.* **2**, 37-85.

—— (1949). On the classification of the Tylenchida, new order (Nematoda, Phasmidia). *Proc. helm. Soc. Wash.* **16**, 37-73.

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Nematologica 6 (1961): 64-68. Leiden, E. J. Brill

OBSERVATIONS ON THE SUSCEPTIBILITY OF CHRYSANTHEMUM VARIETIES INFESTED AT TWO DIFFERENT TIMES WITH CHRYSANTHEMUM EELWORM, *APHELENCHOIDES RITZEMABOSI*

BY

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The susceptibilities of different varieties of chrysanthemum to *Aphelenchoides ritzemabosi*, assessed from a field experiment in 1959, were confirmed by repetition of the experiments at two different sites in 1960 when there was a higher seasonal rainfall than in the previous year. Many plants infested in March were subsequently destroyed by eelworm whereas infestations in August had no effect on the crop of blooms although eelworm symptoms were present. In resistant varieties infested in August there was no spread of symptoms because the infested leaves were killed before the eelworms could multiply and spread. Cuttings of resistant varieties, which are hypersensitive to eelworm attack, were often killed.

Hesling & Wallace (1960) showed that there were differences in susceptibility to eelworm between varieties of chrysanthemum. The differences were found in the dry season of 1959 (Rothamsted rainfall, May to September inclusive, 22.2 cm) and, because weather conditions influence the degree of eelworm infestation, some of the varieties were retested in the wetter season of 1960 (Rothamsted rainfall May to September inclusive, 35.8 cm). To study the effect of early and late infestation, the plants were inoculated when they were either unrooted cuttings in March or well grown plants with flower buds in August. Uninfested plants were controls. Similar experiments were done at Rothamsted and at a commercial nursery at Bell Bar, Hatfield.

MATERIALS AND METHODS

Forty-eight eelworm-free cuttings were taken from hot-water treated stools in each of six chrysanthemum varieties of different susceptibility (Table I). About 100 eelworms were added on 23 March 1960 to each of sixteen unrooted cuttings in each variety using the glass tube method (Hesling & Wallace, 1961). Infested and clean cuttings were rooted in separate boxes of a sterilised loam-sand mixture and care was taken to prevent subsequent accidental infestation of the plants. When rooted, the cuttings were each transferred to a 8.9 cm diameter flower pot and planted outside on 17 May, 1960. At Rothamsted the plants were spaced 76 cm apart and randomised in blocks of varieties. At Bell Bar the spacing had to be closer (30.5 × 38 cm) so similarly treated plants were

TABLE I

Chrysanthemum varieties of different susceptibility to eelworm, tested in 1960.

Pennine Snow	Susceptible
Bill Walters	„
Evelyn Bush	Intermediate
Bill Speat	„
Orange Beauty	Resistant
Orange Peach Blossom	„

placed together in their varieties with a gap of 76 cm between groups. The late infestation of plants was done by the polythene strip method (Hesling & Wallace 1961). 150 eelworms were added to a single leaf on eight of the sixteen uninfested plants at Rothamsted and 300 eelworms were added similarly at Bell

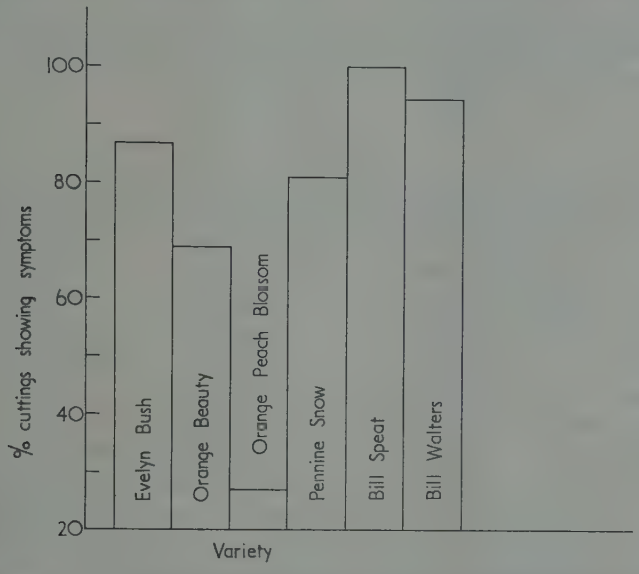

Fig. 1. Percentage of 3 weeks-old plants of different varieties of chrysanthemums showing eelworm symptoms after inoculation with 100 eelworms before rooting.

Bar. The degree of infestation was assessed by counting the number of cuttings and mature plants showing eelworm symptoms and, at the end of the season, the severity of the disease in each variety was assessed subjectively on gross symptoms.

RESULTS

Fig. 1 shows the first assessment of the rooted cuttings before they were potted. Clear symptoms were much rarer on cuttings of Orange Peach Blossom, but infested cuttings of this variety took longer to root than controls and some died, apparently from fungal attack.

When the plants were put outside many showed severe eelworm symptoms but they grew well and soon the symptoms were no longer obvious. In early August after a cool, wet July, leaf-blotch symptoms were noticed and the extent of the disease was assessed. Two further assessments were made at about monthly intervals (Fig. 2). Early infested plants of varieties Pennine Snow and Bill Walters were destroyed by October, whereas Orange Peach Blossom showed little effect. Thus, the widely different susceptibilities of these varieties to eelworm were confirmed. Uninfested plants inoculated with eelworms in August when other plants were showing leaf-blotch symptoms, showed only slight damage and one month elapsed before the disease began to spread up the plants. The different inoculum levels at Rothamsted and Bell Bar produced similar amounts of disease. On almost every plant the late leaf-infestations produced eelworm symptoms. In some varieties the leaf died and the infestation could not be detected at a later examination, suggesting that the infestation had killed the leaf before the eelworms could multiply and spread. This was most noticeable on Orange Peach Blossom, Orange Beauty, Evelyn Bush and Bill Walters.

DISCUSSION

The results confirm earlier findings (Hesling & Wallace 1960) that chrysanthemum varieties differ greatly in their susceptibility to eelworm. They also show that when chrysanthemums are infested as cuttings the eelworm disease that appears in the autumn may be so severe that the plants are destroyed. When clean plants are infested in the late summer there is no indication of spread of the disease for one month and even on susceptible varieties the subsequent disease seems to have no effect on the crop of blooms.

Variety Orange Peach Blossom is very sensitive to eelworm attack and responds by rapid browning of the tissues (Wallace 1960). This reaction may account for the difficulties experienced when rooting infested cuttings of this variety. Necrotic lesions in cuttings infested with eelworms may favour fungal attack and cause further loss of cuttings while they are growing roots, but further work on this using different levels of eelworm inoculum is needed.

The failure of late infestations to spread quickly and seriously to damage the plants suggests that plants almost destroyed by eelworm in the autumn must have been infested at an early stage and also that eelworms are inactive during the summer when dry conditions prevail and there is insufficient free water in the leaves to allow the eelworms to feed, multiply and spread.

Late infestations may easily be overlooked, giving the impression that the plants are eelworm free. Such infestations can recur, for example, from infested weeds or when infested leaves are dropped on to clean plants when blooms are being cut and taken away. In this experiment an inoculum of 100 eelworms to cuttings of susceptible varieties destroyed the plants by the autumn, suggesting that lower numbers of eelworms might produce severe symptoms. This emphasises the need

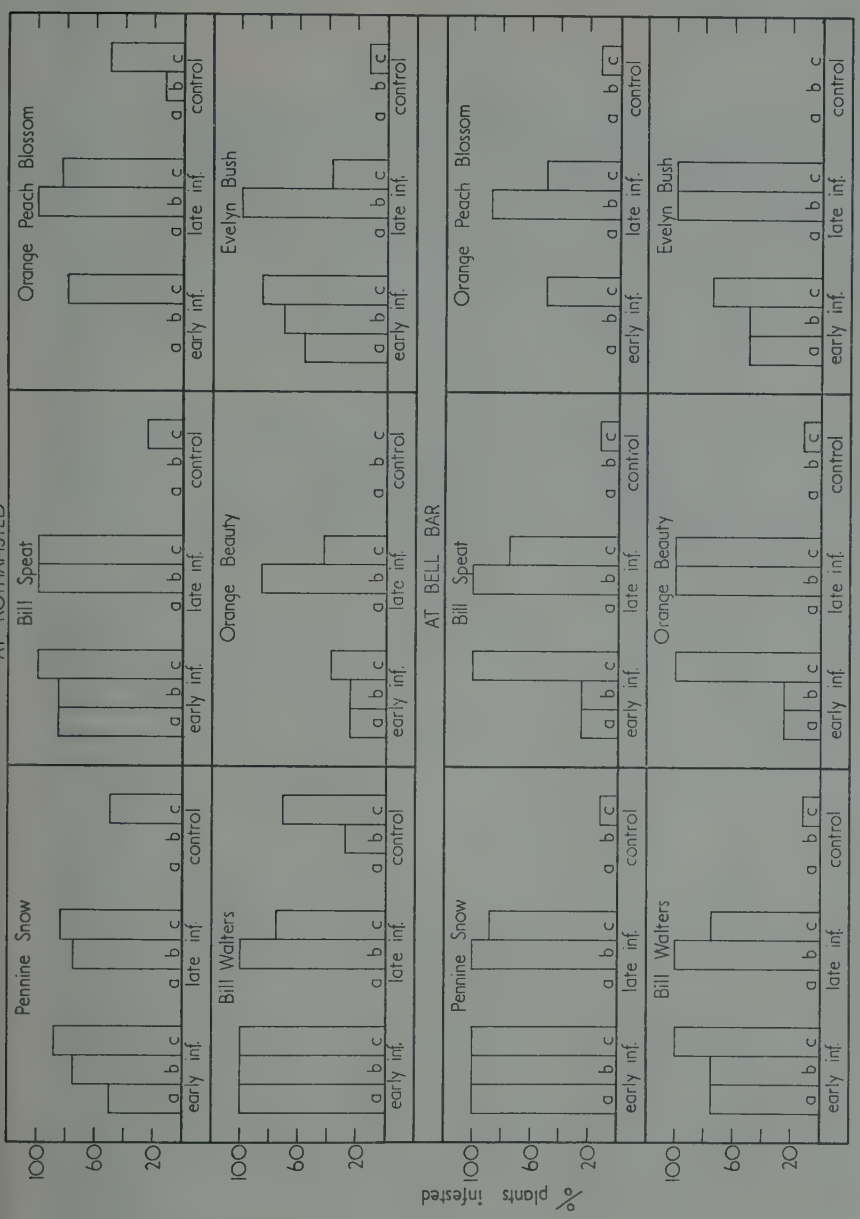

Fig. 2. Percentage of plants of different varieties of chrysanthemum showing symptoms after early or late infestation with eelworms. The plants were examined in August (a), September (b), and October (c). The columns represent percentage of infested plants only and do not indicate the severity of the disease on the plants. inf. = infestation

for complete control of eelworms in the tissues of rudimentary cuttings on the chrysanthemum stool.

ZUSAMMENFASSUNG

Beobachtungen über die Anfälligkeit von Chrysanthemumsorten, die zu verschiedenen Zeiten von Chrysanthemumälchen, Aphelenchoides ritzemabosi, *befallen werden*

Die Anfälligkeit verschiedener Chrysanthemumsorten gegenüber *Aphelenchoides ritzemabosi*, die aus einem Feldversuch 1959 stammten, wurde durch Wiederholung der Versuche an zwei verschiedenen Stellen 1960 bestätigt, wo grössere Niederschläge als im Vorjahr fielen. Manche der im März befallenen Pflanzen wurden durch Älchen später vernichtet, während Befall im August keinen Einfluss auf die Blüte hatte, obwohl Älchensymptome vorhanden waren. Bei resistenten Sorten, die im August befallen wurden, gab es keine Ausbreitung der Symptome, weil die befallenen Blätter zugrunde gingen, bevor die Älchen sich vermehren und ausbreiten konnten. Stecklinge resistenter Sorten, die überempfindlich gegenüber dem Älchenbefall sind, gehen öfters ein.

REFERENCES

HESLING, J. J. & WALLACE, H. R. (1960). Susceptibility of varieties of chrysanthemum to infestation by *Aphelenchoides ritzemabosi* (Schwartz). *Nematologica* **5**, 297-302.

—— & —— (1961). Observations on the biology of the chrysanthemum eelworm, *Aphelenchoides ritzemabosi* (Schwartz) Steiner in florists' chrysanthemum. II. Symptoms of eelworm infestation. *Ann. appl. Biol.* **49**, 204-209.

WALLACE, H. R. (1961). The nature of resistance in chrysanthemum varieties to *Aphelenchoides ritzemabosi*. *Nematologica* **6**, 49-58.

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Nematologica 6 (1961): 69-84. Leiden, E. J. Brill

ANZIEHUNGSVERSUCHE MIT *DITYLENCHUS DIPSACI* UNTER BERÜCKSICHTIGUNG DER WIRKUNG DES KOHLENDIOXYDS, DES REDOXPOTENTIALS UND ANDERER FAKTOREN

VON

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In einer früheren Arbeit haben wir berichtet, dass Kohlendioxyd — vermittels einer Kapillare in Erde eingeleitet — auf *D. dipsaci* anziehend wirkt. Es wurden neue Versuche in Agar angelegt, um den Einfluss des Redoxpotentials, des pH und anderer Faktoren zu studieren. Die Anziehung zu CO_2-Quellen wird bestätigt. Diese erfolgte in Abwesenheit von pH- und Redoxpotential-Gradienten. Die Hypothese von der anziehenden Wirkung eines erniedrigten Redoxpotentials trifft für *D. dipsaci* nicht zu. Die Wirtsfindung dieser Art wird auf Grund der erzielten Resultate diskutiert.

Über die Anziehung von Nematoden zu Wirtspflanzen liegen ziemlich viele Untersuchungen vor. Darin gehen bezüglich der Ursachen einer Anziehung bezw. Ansammlung die Ansichten zum Teil auseinander. Es gibt Arbeiten, welche eine anziehende Wirkung der Wirtswurzeln verneinen, oder den Ausscheidungen der Pflanze hauptsächlich eine bloss aktivierende Wirkung auf die Nematoden zuschreiben (Kühn, 1959; Weischer, 1959). Nach Kühn kommt keine gerichtete Wanderung vor, während Weischer aber in unmittelbarer Wurzelnähe eine solche vermutet.

Aus den Arbeiten anderer Autoren geht hervor, dass sie eine Anziehung, d.h. eine Lockwirkung des Wirtes annehmen. Ob die dadurch verursachten Ansammlungen durch gerichtete oder ungerichtete Orientierungsreaktionen zustande kommen, ist in diesen Fällen nicht untersucht worden. Hierher gehören die Publikationen von Baunacke (1922), Linford (1939), Gadd & Loos (1941), Wieser (1955 und 1956), Wallace (1958).

Wenn aus den meisten zitierten Arbeiten auch hervorgeht, dass eine Anziehung der Nematoden zu den Wirtswurzeln stattfindet, so sind bezüglich der chemischen Zusammensetzung der Lockstoffe keine Aussagen gemacht. Gewisse Publikationen gestatten aber die Vermutung, dass es sich bei den anziehenden Faktoren nicht um spezifische handelt. So konnten Gadd & Loos (1941) Anziehung zu Nichtwirtspflanzen feststellen. Dickinson (1959) kommt bei *Heterodera schachtii* zur Auffassung, dass sich Spezifität für besondere Wirtswurzeln erst zeigt, wenn die Wurzel erreicht und der Stachel eingestossen ist. Die Experimente von Shepherd (1959) endlich suggerieren, dass die Larven verschiedener *Heterodera*-Arten in

alle Wurzeln eindringen, mit denen sie in Kontakt kommen, auch wenn sich diese nicht als Nahrung eignen.

Zur Ermittlung der für die Anziehung der Nematoden in Frage kommenden Faktoren können im weiteren selbstverständlich gewisse Orientierungsarten, wie z.B. die optische, a priori ausgeschlossen werden. Ohne Bedenken darf man auch annehmen, dass Feuchtigkeits- und Temperaturgradienten für das Auffinden von Wurzeln kaum von Bedeutung sind. Hingegen ist es naheliegend, eine Orientierung nach Gradienten von Wurzelausscheidungen und Wurzelzerfallsprodukten in Betracht zu ziehen. Diese können gasförmig auftreten oder im Bodenwasser gelöst sein. Es ist denkbar, dass gasförmige *und* gelöste Stoffe vom Tier für die Orientierung ausgewertet werden.

Vor kurzem hat Bird (1959) für die Anziehung zu den Wurzeln einen weiteren Faktor in die Diskussion geworfen. Er stellte die Hypothese auf, dass pflanzenparasitäre Nematoden einem Potentialgradienten in Richtung des negativen Potentials folgen, der durch die Erniedrigung des Redoxpotentials entsteht. Tatsächlich weisen die lebenden Pflanzenwurzeln gegenüber dem umgebenden Boden eine negative Potentialdifferenz auf. Der Sitz dieser Potentialdifferenz ist hauptsächlich die Grenzfläche zwischen Wurzel und umgebender Lösung (Lundegårdh, 1940); die Reichweite des Potentialgefälles ist damit als gering anzunehmen.

Hinsichtlich der Wurzelausscheidungen bestand lange Zeit wenig Klarheit. Unbestritten war lediglich die Tatsache der gasförmigen Kohlendioxyd-Abgabe. Heute ist eine grosse Zahl von Stoffen nachgewiesen, darunter viele Aminosäuren, die von den Wurzeln abgeschieden werden (Katznelson, Rouatt & Payne, 1955; Rovira, 1956 & 1959). Es war jedoch nicht immer möglich, exakt zu unterscheiden zwischen tatsächlicher Ausscheidung und Freilegung durch Autolyse von Wurzelteilen, z.B. abgestossenen Wurzelhaaren. Für die Anziehung zu den Wurzeln scheint uns diese Tatsache aber nicht von grosser Bedeutung.

Thorpe, Crombie, Hill & Darrah haben 1947 nachgewiesen, dass einige der abgeschiedenen Aminosäuren (z.B. Glutaminsäure und Asparaginsäure) bei Drahtwürmern eine Orientierungsreaktion auslösen. Bird (1959) erzielte mit Glutaminsäure ebenfalls eine Anziehung bei Nematoden. Diese Aminosäuren sind nicht als spezifisch für bestimmte Pflanzen anzusehen.

Die mengenmässig wichtigste von den lebenden Wurzeln abgegebene Ausscheidung ist das gasförmige Kohlendioxyd. Dank dieser Tatsache und dem ausgezeichneten Diffusionsvermögen ist dieses Gas sicher in der Lage, im Boden einen Gradienten über eine gewisse Distanz zu bilden und aufrechtzuerhalten. Die übrigen Ausscheidungen sind in viel geringerer Menge vorhanden und eine Gradientbildung ist daher höchstens in unmittelbarer Wurzelnähe denkbar. Offenbar wegen seiner Nicht-Spezifität wurde aber das CO_2-Gas bei Orientierungsversuchen bis vor kurzem ausser acht gelassen.

Versuche über die anziehende Wirkung von natürlichen und künstlichen Kohlendioxyd-Quellen ergaben mit verschiedenen bodenbewohnenden Insekten posi-

tive Resultate (Klingler, 1957, 1958); Versuche mit einer Nematodenart (*Ditylenchus dipsaci* (Kühn)) führten zu ebensolchen Ergebnissen (Klingler, 1959). Voraussetzung für die Erzielung einer Anziehung in allen diesen Fällen war die Aufrechterhaltung eines in biologischen Grenzen liegenden gasförmigen CO_2-Gradienten und eine im übrigen normale Sauerstoffversorgung der Versuchstiere. Bird (1959) erhielt mit seiner Methode (ohne direkte Gaseinleitung) keine Anziehung von *Meloidogyne spp.* zu CO_2, korrigierte sich aber 1960 (siehe Nachtrag p. 82).

Die vorliegende Arbeit bestätigt unsere frühere Beobachtung über die anziehende Wirkung von Kohlendioxyd auf *D. dipsaci.* Sie verfolgt den Zweck, die Hypothese von Bird an dieser Art zu prüfen und ferner zu untersuchen, ob die Anziehung durch Kohlendioxyd über eine Änderung des Redoxpotentials oder eventuell des pH erfolge.

Die Möglichkeit, dass CO_2-Gradienten *und* Redoxpotential-Gradienten eine anziehende Wirkung ausüben, würde keinen Widerspruch in sich schliessen. Das Gegenteil ist der Fall. Nach Lundegårdh (1940) koinzidieren hohe negative Oberflächenpotentiale der Wurzeln und hohe metabolische Aktivität. Hohe metabolische Aktivität bedeutet aber in diesem Falle auch starke Respiration und hohe CO_2-Bildung. Es tritt also eine parallele Entwicklung zwischen negativem Wurzelpotential und CO_2-Bildung auf. Auf Grund dieser Feststellung können die Resultate von Linford (1939), die Bird zur Stützung seiner Hypothese angeführt hat, auch als Bestätigung der anziehenden Wirkung des Kohlendioxyds angesehen werden.

VERSUCHSTECHNIK

Die für unsere Versuche verwendeten Nematoden der Art *Ditylenchus dipsaci* wurden in grossen Mengen aus infizierten Zwiebeln und Lauch gewonnen, zum kleinen Teil auch durch Extraktion aus befallenen Zuckerrüben.

In allen Versuchen wurde mit Agar-Nematodensuspensionen gearbeitet, in enger Anlehnung an die von Bird (1959) angewendete Methodik. Wir verwendeten ebenfalls Difco-bacto-Agar, der 2%ig hergestellt und nach Abkühlung auf ca. 45° C mit der gleichen Menge einer Wasser-Nematodensuspension auf 1% verdünnt und dadurch zugleich etwas abgekühlt wurde. Diese noch flüssige Mischung konnte dann in Petrischalen oder ähnliche Formen gegossen werden.

Anziehungsexperimente wurden mit Gasen (CO_2, O_2, H_2), mit einem Reduktionsmittel (Natriumdithionit) und einem Oxydationsmittel (Kaliumpermanganat in saurem Milieu) durchgeführt. Ferner wurde auch mit elektrischem Strom von verschiedener Spannung gearbeitet. In den meisten Fällen wurde die Frage nach der Anziehung im Zusammenhang mit den entsprechenden Redoxpotentialen untersucht.

Die Gase wurden aus der mit Reduzier- und Nadelventil versehenen Bombe durch Gummischlauch und Glaskapillare in den Agar eingeleitet. Oxydations- oder Reduktionsmittel einer bestimmten Konzentration (z.B. 0,2 m) wurden mit

der gleichen Menge 2%igem Agar von ca. 45° C gemischt, sodass als Resultat 1% Agar und die halbe Konzentration des Reduktionsmittels (also 0,1 m) resultierte. Wenn dieser Agar erstarrt war, konnte mit einem Glasröhrchen ein Pfropfen herausgestochen werden; dieser Pfropfen (Ø je nach Versuch 3-5 mm) wurde in eine Petrischale gelegt und dann die oben erwähnte Agar-Nematodensuspension darum herumgegossen.

Bei den Versuchen mit elektrischem Strom tauchten zwei Kupfer- oder Platindrähte als Elektroden in den Agar ein.

Das Resultat der Anziehungsversuche wurde nur in wenigen Fällen durch quantitative Auszählung ermittelt; in den meisten Fällen wurde es einfach auf Grund mikroskopischer Beobachtung beurteilt.

Redoxpotential- und pH-Messung, Pufferung des Agar

Die Messung des Oxydations-Reduktions-Potentials (ORP) erfolgte mit einem auch für diesen Zweck eingerichteten pH-Meter. Als Messelektrode diente eine Platinelektrode mit planer Messfläche von ca. 5 mm Ø. Diese wurde in die zu messende Stelle des Agars leicht eingetaucht. Als Bezugselektrode stand eine gesättigte Kalomelelektrode zur Verfügung.

Bei der Messung des Redoxpotentials ging es weniger um die absoluten Werte als vielmehr um die Potential*differenzen.*

Für die Messung der Wasserstoffionen-Konzentration (pH) verwendeten wir eine Glaselektrode mit ebenfalls planer Messfläche von ca. 5 mm Ø. Der Agar wurde in den meisten Versuchen gepuffert. Als Puffer fand eine Zitronensäure-sekundäre Natriumphosphat-Lösung Verwendung. Das pH wurde im Gebiet von etwa 5,1 bis 5,2 eingestellt. Es wurde vor allem so tief gewählt, um der Senkung des pH durch das Reduktionsmittel und auch durch das Kohlendioxyd entgegenzukommen. Durch die Nivellierung auf einen bestimmten Wert konnte das pH als Versuchsfaktor ausgeklammert werden. Da ferner das Redoxpotential pH-abhängig ist, konnte durch die Pufferung dieser Einfluss für alle Messungen eines Versuches konstant gehalten werden. — Einzelheiten der Versuchstechnik werden in den speziellen Kapiteln behandelt.

ANZIEHUNGSVERSUCHE MIT GASEN

Kohlendioxyd

Die Versuche mit Kohlendioxyd wurden nach zwei Methoden durchgeführt. Nach der ersten Methode wurde eine Agarscheibe (mit Nematoden in Suspension) von ca. 6 cm Ø auf ein feines Drahtgeflecht gelegt. Die CO_2-führende Glaskapillare kam *auf* diese Agarscheibe zu liegen und ragte vom Rand her etwa 2 cm einwärts. Über die Agarscheibe und Kapillare wurde schliesslich eine Glasplatte gelegt, die das ganze zudeckte. Das Drahtgitter als Unterlage gestattet eine gewisse natürliche Belüftung des Agars von unten, jedenfalls konnte auf diese Weise Immobilisierung durch Sauerstoffmangel vermieden werden.

J. KLINGLER: *Anziehungsversuche mit* Ditylenchus dipsaci.

Abb. 1. Ansammlung von *D. dipsaci* um die Mündung einer CO_2-führenden Kapillare (Methode 1).

J. KLINGLER: *Anziehungsversuche mit* Ditylenchus dipsaci.

Abb. 2. Ansammlung um die Mündung einer CO_2-führenden Kapillare (nach Methode 2; gestattet pH- und ORP-Messungen).

Die beschriebene Versuchsanlage gestattete sehr gut die Bildung eines Gradienten. Es muss aber dafür gesorgt werden, dass überschüssiges Kohlendioxyd — am besten der Kapillare entlang — nach aussen entweichen kann, weil sonst störende Blasen auftreten. Die eingeleitete CO_2-Menge muss trotzdem sehr klein sein, etwa 0,5 ml/h.

Die Resultate mit dieser Methode waren sehr gut. Die Ansammlungen im Gebiet der Kapillarmündung nahmen im Laufe von wenigen Stunden ein Ausmass an, wie es die Abb. 1 sehr schön zeigt. Der grosse Nachteil dieser Methode lag darin, dass keine ORP- und pH-Messungen während des Versuchsablaufes möglich waren.

Die zweite Methode trug diesem Umstand Rechnung. Hier wurde die Agar-Nematodensuspension in eine Petrischale von ca. 8 cm Ø gegossen und das Kohlendioxyd einfach durch eine von schräg oben eintauchende Glaskapillare eingeleitet. Das überschüssige Gas muss auch hier entweichen können, z.B. der Kapillare entlang nach oben, oder durch einen feinen in den Agar eingestochenen Kanal von der Mündung senkrecht nach oben. Da bei dieser Methode der Agar oben durch keine Glasplatte zugedeckt ist und das Gas sehr leicht entweichen kann, dürfen hier etwas grössere CO_2-Mengen einströmen, bis 10 ml/h. Die natürliche Luftsauerstoffversorgung der Nematoden bei dieser Versuchsanordnung ist nicht in Frage gestellt.

Die Resultate nach dieser zweiten Methode waren ebenfalls eindrücklich positiv. Deutliche, von Auge sichtbare Ansammlungen konnten schon nach einer Stunde beobachtet werden (Abb. 2). Nach dem Unterbruch der CO_2-Zufuhr löste sich eine Ansammlung innerhalb einer viertel bis halben Stunde vollkommen auf. Bei grossen CO_2-Mengen ($>$ 10 ml/h) bildete sich direkt um die Mündung eine nematodenfreie Zone von wenigen Millimetern Durchmesser. Dies deutet auf Abstossung infolge zu hoher CO_2-Konzentration. Um die nematodenfreie Zone herum kam es aber zu sehr kräftigen, ringförmigen Ansammlungen.

Die Messung des ORP und des pH konnte direkt über der Mündung (Abb. 3) und in jedem beliebigen Abstand davon vorgenommen werden. Allfällige Gradienten waren auf diese Weise messbar. Zur Kontrolle wurde auch immer das pH gemessen, denn geringe Veränderungen desselben waren trotz der Pufferung denkbar. Die Messungen in den Versuchen mit Kohlendioxyd und gepuffertem Agar ergaben aber für das *pH* immer ein ausgeglichenes Niveau, d.h. die gleichen Werte bei der Mündung wie in einem beliebigen Abstand davon. Dieses Niveau entsprach dem durch die Pufferung herbeigeführten Wert von etwa pH 5,2. *Wir können also feststellen, dass die Anziehung zur CO_2-Quelle ohne pH-Gradient erfolgte.*

Bird (1959) hat festgestellt, dass seine Nematoden nicht von speziellen Wasserstoffionenkonzentrationen angezogen wurden. Die Ergebnisse decken sich also in diesem Punkt.

Die Messungen des *Redoxpotentials* bei der Kapillar-Mündung und in verschiedenen Abständen davon ergaben — erwartungsgemäss — keine Beeinflus-

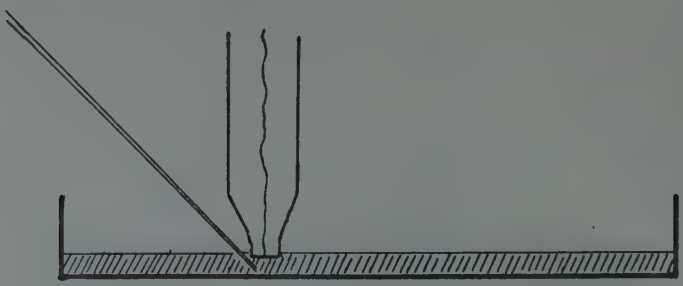

Abb. 3. Position der Messelektrode bei der Mündung zur Messung des ORP (oder auch des pH).

sung dieser Werte durch eine künstliche attraktive CO_2-Quelle. Mit anderen Worten: *Die Anziehung der Nematoden zur CO_2-Quelle erfolgte ohne Potentialgradient*. Daraus lässt sich schliessen, dass eine Anziehung zu den Wurzeln einer Wirtspflanze auch ohne Potentialgefälle möglich ist. Nachdem also die Anziehung zu einer künstlichen Kohlendioxyd-Quelle ohne pH- und ORP-Gradient vor sich gehen kann, darf die Vermutung geäussert werden, dass eine direkte spezifische Wahrnehmung dieses Gases durch *D. dipsaci* wahrscheinlich ist.

Die Resultate der Redoxpotentialmessungen aus drei Versuchen, alle mit sehr guter Anziehung (wie z.B. Abb. 2) geben wir nachstehend in Tabelle I wieder. Das pH aller dieser Versuche war konstant ($\approx$ 5,2).

Aus Tabelle I ist ersichtlich, dass die künstlichen CO_2-Quellen, wie erwartet, keine Potentialgradienten verursachten. Abweichungen der Messwerte um $\pm$ 10

Tabelle I

Ergebnisse von Redoxpotentialmessungen in Versuchen mit Kohlendioxyd

Versuch Nr.	Abstand der Messung von Kapillarmündung	mV-Ablesung (E. Kal.)	ORP-Differenz gegenüber Mündung
	0 cm	+ 125 mV	
I	2 cm	+ 130 mV	+ 5 mV
	4 cm	+ 130 mV	+ 5 mV
	0 cm	+ 120 mV	
II	3 cm	+ 110 mV	— 10 mV
	4 cm	+ 130 mV	+ 10 mV
	0 cm	+ 134 mV	
III	2,5 cm	+ 136 mV	+ 2 mV
	5,5 cm	+ 134 mV	0 mV

mV können unter den beschriebenen Versuchsbedingungen nicht als signifikant angesehen werden.

Sauerstoff

Die Versuche mit Sauerstoff wurden genau gleich angelegt, wie es unter Methode 2 des vorigen Kapitels beschrieben ist. Es strömten ähnliche O_2-Mengen in den Agar ein, wie in den vorigen Versuchen CO_2, und es wurde ebenfalls für das Entweichen des überschüssigen Gases gesorgt.

Sämtliche Versuche mit Sauerstoff verliefen *negativ*, es trat keine Anziehung und keine Ansammlung auf. In gewissen Fällen konnte eher eine Tendenz zur Meidung der näheren Umgebung der Mündung festgestellt werden. Vereinzelte Beobachtungen lassen es andererseits nicht als ausgeschlossen erscheinen, dass unter O_2-Mangel-Bedingungen Ansammlungen bei einer Sauerstoffquelle vorkommen könnten.

Trotz der negativen Ergebnisse wurden einige Messungen vorgenommen. Diese zeigten weder beim pH noch beim ORP Veränderungen.

Wasserstoff

Die H_2-Einleitung geschah unter anderem in der Absicht, die in den bisherigen Versuchen ausgebliebene Senkung des Redoxpotentials herbeizuführen und die Reaktion der Nematoden auf diese Senkung zu untersuchen. Es wurde mit verschiedenen H_2-Mengen gearbeitet, variierend zwischen 0,5 ml h und 12 ml/h.

In einer ersten Serie von Versuchen gingen wir genau gleich vor wie beim Sauerstoff und beim Kohlendioxyd (Methode 2), d.h. wir liessen einfach das Gas einströmen. Diese Versuche führten alle zu *negativen* Ergebnissen. Es konnten keine Ansammlungen beobachtet werden.

Die Methode 2 bildete auch die Grundlage für eine zweite Serie von Versuchen, doch wurde hier zusätzlich die Platinmesselektrode während des ganzen Versuches über der Kapillarmündung in den Agar eingetaucht (wie es Abb. 3 zeigt). (Erst später wurde für die Messung selbst auch die Kalomelbezugselektrode eingetaucht und das Messgerät eingeschaltet.) Das H_2-Gas strömte also während der ganzen Dauer des Experimentes über das Platinplättchen der Messelektrode (Wasserstoffelektrode).

Durch die beschriebene Versuchsanlage ergab sich bei der Mündung ein gegenüber der weiteren Umgebung stark erniedrigtes Redoxpotential. Diese Erniedrigung variierte in unseren Versuchen zwischen rund 200 bis 400 mV. Das pH des Agars änderte sich nicht, sondern behielt sein übliches Niveau (5,2), auch an der Mündung bei. Trotz des ausgeprägten, gegenüber der Umgebung stark negativen Redoxpotentials konnte in keinem Versuch eine Anziehung oder Ansammlung beobachtet werden. Alle Ergebnisse waren *negativ*. Um die Mündung herum wurde vielmehr nach einiger Zeit ein nematodenfreier Hof von 1-2 cm Ø beobachtet. Ansammlungen waren auch ausserhalb dieses Hofes nicht wahrzunehmen.

Demnach ergab also eine durch Wasserstoff herbeigeführte Erniedrigung des Redoxpotentials keine Anziehung von *D. dipsaci*.

REDUKTIONS- UND OXYDATIONSMITTEL

Es wurden als Reduktionsmittel Natriumdithionit ($Na_2S_2O_4$) und als Oxydationsmittel Kaliumpermanganat ($KMnO_4$) verwendet.

Reduktionsmittel

Anfängliche Versuche mit Natriumdithionit nach der Methode von Bird (1959) (ein Pfropfen Agar/Natriumdithionit mit Agar/Nematoden-Suspension umgossen) wurden in ungepuffertem Agar durchgeführt.

Sehr gute Anziehung ergaben Konzentrationen von 0,5 m und 0,1 m Na-dithionit. Bei 0,01 m Konzentration fiel die Wirkung bereits deutlich ab. Das 0,5 m Na-dithionit wirkte allerdings toxisch, erkennbar daran, dass die Ansammlungen sich später nicht mehr auflösten und nur noch aus längsgestreckten, toten Nematoden bestanden. Diese Ergebnisse stimmen mit jenen Bird's allgemein überein.

Nicht zuletzt weil das verwendete Reduktionsmittel das pH des Agars stark senkte, wurde in den späteren Versuchen der Agar auf das ziemlich tiefe Niveau von pH 5,1 bis 5,2 gepuffert. *Ohne* Pufferung ergab sich ein pH-Gradient, welcher im 0,1 m Agar/Natriumdithionit-Pfropfen einen um 2,2 Einheiten tieferen pH-Wert erreichen konnte als in der Umgebung. Durch die Pufferung konnte diese Erniedrigung zwar nicht ganz eliminiert, aber immerhin auf eine Differenz von bloss ca. 0,3 pH vermindert werden. Selbstverständlich wurde der Agar/Natriumdithionit-Pfropfen in gleicher Weise gepuffert wie die umgebende Agar/Nematoden-Suspension. Die Ergebnisse dieser Versuche waren ebenfalls positiv. Obschon sie nicht quantitativ erfasst wurden, herrschte aber der Eindruck vor, dass die Anziehung etwas weniger deutlich ausfiel als im ungepufferten Agar.

Zwei mit ¼ m Natriumdithionit-Konzentration durchgeführte Versuche gaben eine deutliche Ansammlung innerhalb weniger als 1½ Stunden. Eine Kontrolle nach etwas weniger als 3 Stunden zeigte bereits beide Ansammlungen in Auflösung begriffen, und nach 5 Stunden waren keine solche mehr wahrzunehmen. Zu diesem Zeitpunkt ergab aber eine Messung noch eine starke negative Redoxpotentialdifferenz im Pfropfen gegenüber der weiteren Umgebung (rund 450 mV im einen und 370 mV im zweiten Versuch). Die Auflösung erfolgte also trotz dieses noch bestehenden negativen Potentialgradienten.

Ein dritter Versuch mit ¼ m Na-dithionit verlief ergebnislos. Die Ursache dieses Misserfolges ist unbekannt. Zwei Versuche mit 0,1 m Natriumdithionit zeigten bei der Kontrolle nach drei Stunden eine sehr deutliche Ansammlung. Die ORP-Messung, die in einem dieser Versuche bereits nach einer Stunde vorgenommen wurde, ergab im Pfropfen eine gegenüber der Umgebung sehr starke negative Differenz von über 500 mV. Die festgestellten Ansammlungen lösten sich dann

bis zum andern Morgen vollständig auf. Es waren aber in diesem Zeitpunkt der Auflösung, 17 Stunden nach Versuchsbeginn, noch deutliche negative Potentialdifferenzen vorhanden, nämlich 85 mV im ersten und 75 mV im zweiten Versuch. Die Auflösung der Ansammlungen geschah also auch hier wieder zu einer Zeit, da noch negative Potentialgradienten vorhanden waren. Es scheint also,, dass die Anziehung in keiner direkten Beziehung zum Potentialgradienten stand.

Oxydationsmittel

Die Versuche mit dem Oxydationsmittel Kaliumpermanganat (in saurem Milieu) wurden durchgeführt, um zu prüfen, ob durch eine *Erhöhung* des Redoxpotentials eine Abstossung der Nematoden eintritt, wie dies beim Zutreffen der

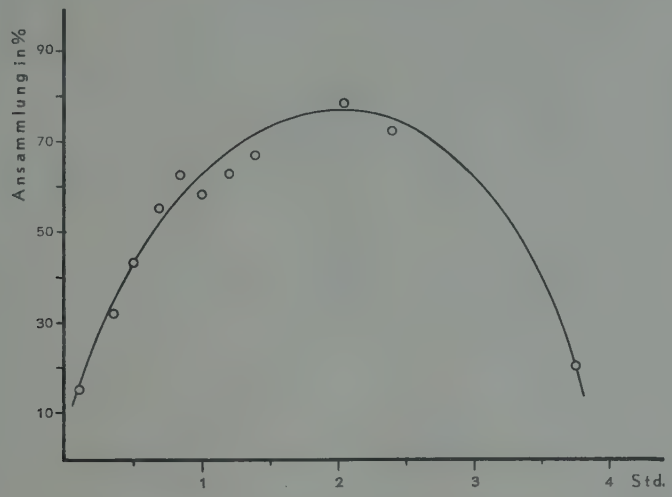

Abb. 4. Verlauf der Ansammlung und Wiederauflösung derselber in einem Versuch mit dem Oxydationsmittel Kaliumpermanganat; 0,1 m.

Hypothese von Bird erwartet würde. Die Versuchsmethode war die gleiche wie für das Reduktionsmittel. Die verwendeten Agar-$KMnO_4$-Propfen wurden in zwei verschiedenen Kaliumpermanganat-Konzentrationen geprüft, 0,1 m und 0,05 m. Wie erwartet, ergab sich durch dieses Oxydationsmittel ein gegenüber der Umgebung deutlich erhöhtes Redoxpotential, zugleich stieg aber auch das pH um mehr als eine Einheit an. Trotz der positiven Redoxpotentialdifferenz konnten in diesen Versuchen nach 1-2 Stunden deutliche Ansammlungen festgestellt werden. Es trat also Anziehung auf.

Bei zwei parallel geführten Versuchen mit einer ca. 0,05 m Kaliumpermanganat-Konzentration konnte bereits nach 1 Stunde eine starke Ansammlung direkt um den Pfropfen herum beobachtet werden. Nach zwei Stunden zeigte sich noch

die gleiche Stärke der Ansammlung, mit zugleich intensiver Einwanderung in den Pfropfen selbst. Das Redoxpotential und das pH dieser Versuche wurde nach 2½ Stunden gemessen. Das Messgerät zeigte im Agar/$KMnO_4$-Pfropfen einen gegenüber der weiteren Umgebung um rund 70 mV erhöhten Wert. Das pH war ebenfalls angestiegen, und zwar von 5,2 auf 6,5. (Hier ist zu beachten, dass unter sonst gleichen Bedingungen eine Zunahme des pH eine Abnahme des ORP zur Folge hat. Auf den gleichen pH-Wert bezogen, würde also die obige Potentialdifferenz von 70 mV effektiv noch grösser ausfallen.)

In einem der mit 0,1 m Kaliumpermanganat durchgeführten Versuche wurde der quantitative Verlauf der Ansammlung durch Auszählung verfolgt (Abb. 4). Der 0,1 m Kaliumpermanganat/Agar-Pfropfen von 2 mm Ø befand sich in diesem Versuch an exzentrischer Lage in einer Agarscheibe von 24 mm Ø. Ausgezählt wurden in Intervallen alle Nematoden in einer Kreisfläche von 8 mm Ø mit dem Pfropfen als Zentrum, sowie einmalig die Gesamtzahl der Nematoden in der ganzen Agarscheibe, in diesem Versuch 120 an der Zahl. Aus diesen beiden Zahlen resultierte die prozentuale Ansammlung. Die als Mass für die Ansammlung ausgezählte Kreisfläche von 8 mm Ø entsprach rund 11 % der Gesamtfläche. Bei zufälliger Wanderung wären also durchschnittlich 11 % der Nematoden in dieser Kreisfläche zu erwarten gewesen, tatsächlich wurden jedoch bis gegen 80 % gefunden.

Zusammenfassend können wir zu den Versuchen mit dem Oxydationsmittel Kaliumpermanganat sagen, dass in Bezug auf Redoxpotential und pH die Verhältnisse genau umgekehrt lagen wie beim Reduktionsmittel Natriumdithionit. Trotzdem kam es in beiden Fällen zu klarer Anziehung. Dies spricht gegen die Redoxpotential-Hypothese. Welche Eigenschaft dieser beiden anorganischen Verbindungen allerdings für die Anziehung verantwortlich zu machen ist, darüber wissen wir vorläufig nichts. Die naheliegende Vermutung, dass zwar nicht die Erniedrigung, wohl aber einfach eine Veränderung des Redoxpotentials wirksam sei, erweist sich angesichts gewisser Resultate (z.B. mit Wasserstoff) als wenig wahrscheinlich.

VERSUCHE MIT ELEKTRISCHEN STRÖMEN

Die Versuchsanlage wurde sehr einfach gewählt. Der negative und positive Pol eines elektrischen Elementes wurden an je einen Metalldraht angeschlossen, welche als Elektroden von oben in den Agar eintauchten. Bei genügender Spannung treten sichtbare Elektrolysevorgänge auf. Gleichzeitig wird der Agar in der Umgebung der Kathode reduziert (kathodische Reduktion) und an der Anode oxydiert (anodische Oxydation). Dies äussert sich in einer entsprechenden Veränderung des Redoxpotentials des Agars. Ferner wird auch das pH beeinflusst, es erfährt an der Kathode eine Erhöhung und an der Anode eine Erniedrigung. Die Reaktion der im Agar suspendierten Nematoden auf alle diese Vorgänge wurde beobachtet.

Es wurden Spannungen von 4,5 V und 1,5 V sowie von 250 mV angelegt, welche in den ersten beiden Fällen direkt von elektrischen Elementen, im letzten

Fall durch Abgreifen von einem Schieberrheostaten herrührten. Die Versuche erfolgten sowohl in gepuffertem wie in ungepuffertem Agar; als Elektroden dienten in beiden Fällen Kupfer- als auch Platindrähte von 1 mm bezw. 0,6 mm Ø. Der Abstand der beiden Elektroden betrug jeweils etwa 2 cm, in gewissen Fällen bis über 5 cm.

Beobachtungen und Resultate

4,5 Volt: Bei Verwendung von Platindrähten als nicht angreifbare Elektroden konnte Gasbildung sowohl an der Kathode (H_2) wie an der Anode (O_2) beobachtet werden. Die Kupferdrähte erwiesen sich dagegen als angreifbare Elektroden. Es konnte nur an der Kathode Gasbildung beobachtet werden, während an der Anode Kupferionen in Lösung gingen, welche den Agar in der Umgebung blau färbten. Diese Blaufärbung ging im gepufferten Agar rascher und stärker vor sich als im ungepufferten. Der Stromfluss betrug gleich nach dem Eintauchen der Elektroden 0,5 mA im ungepufferten und 2,5 mA im gepufferten Agar.

Die Reaktion der Nematoden war die folgende: Im *ungepufferten Agar* und mit Kupferelektroden zeigten sich nach längerer Versuchsdauer schwache, andeutungsweise Ansammlungen an der Anode. Die Kathode wurde etwas weniger aufgesucht. Mit Platinelektroden konnte dagegen eine deutliche Meidung der Umgebung der Anode festgestellt werden, während gegenüber der Kathode Indifferenz, in gewissen Fällen sogar leichte Bevorzugung beobachtet werden konnte. Es bestehen also Unterschiede zwischen den beiden Elektrodentypen.

Im *gepufferten Agar* und mit Kupferdrähten als Elektroden zeigten sich im Laufe der Versuche schwache ringförmige Ansammlungen um die Kathode. Diese Ringe bildeten sich je nach Versuchsdauer in einem Abstand von etwa 5-8 mm um die Kathode herum. Mit den Platinelektroden ergab sich ebenfalls eine Bevorzugung der Kathode, daneben aber eine deutliche Meidung der Anode.

1,5 Volt: An keiner der Platinelektroden konnte Elektrolyse bezw. Gasbildung beobachtet werden. Bei Verwendung von Kupferdrähten trat hingegen an der Kathode Gasbildung auf, während an der Anode wieder Kupferionen in Lösung gingen, letzteres allerdings im ungepufferten Agar nur in sehr geringem Umfang. Der Stromfluss im ungepufferten Agar mit Platinelektroden lag in der Grössenordnung 7.10^{-8} A, in gepufferten Agar 2.10^{-7} A (gleich nach dem Eintauchen).

In diesen Versuchen bildeten sich direkt um die Kupferanoden herum nach wenigen Stunden kräftige Ansammlungen, die ein bedeutend grösseres Ausmass annahmen als in den übrigen elektrischen Versuchen. Es konnte andererseits keine Meidung der Kathode beobachtet werden. Mit den Platinelektroden ergaben sich wiederum andersartige und weniger einheitliche Resultate. Im ungepufferten Agar konnten in zwei von drei Versuchen leichte Ansammlungen direkt an der Kathode beobachtet werden. Im dritten Versuch trat eine nur schwache Bevorzugung der Anode auf. Im gepufferten Agar wurde an keiner der beiden Platinelektroden eine Ansammlung beobachtet.

200-250 Millivolt: Alle Versuche verliefen negativ. An keiner der Elektroden

kam es zu Ansammlungen. Elektrolytische Vorgänge waren nicht wahrzunehmen, d.h. keine Gasbildung und keine Abgabe von Kupferionen an der Anode. Die Abstände von Kathode und Anode im Agar betrugen in diesen Versuchen rund 1 cm.

Die Versuche mit 200-250 mV-Spannung sind insofern interessant, als sie nur negative Resultate lieferten. Dies ist bemerkenswert, da diese Potentialdifferenzen am wenigsten von den zwischen Wurzeln und Bodenlösung festgestellten abweichen, welche Lundegårdh (1942) unter bestimmten Bedingungen mit etwas über 60 mV angab.

Wir wollen versuchen, aus der Vielfalt der Beobachtungen bei den Experimenten mit 4,5 und 1,5 Volt die Tendenzen herauszustellen. Unterschiedliche Resultate ergaben sich hauptsächlich durch die Verwendung verschiedenartiger Elektroden, ferner durch die Verwendung von gepuffertem und ungepuffertem Agar und von verschiedenen Spannungen.

Im allgemeinen wirkte die angreifbare Kupferanode anziehend. Eine Ausnahme bildeten die Versuche mit 4,5 V in gepuffertem Agar, wo die Kupferanode zwar nicht anziehend, aber auch nicht abstossend wirkte. Die nicht angreifbare Platinanode wurde hingegen vorwiegend deutlich gemieden.

Der Unterschied in der Wirkung der beiden Elektroden kann nicht in der elektrischen Ladung liegen, da es sich in beiden Fällen um Anoden handelt. Ein wichtiger Unterschied liegt dagegen darin, dass die Kupferanode Kupferionen in den Agar abgibt und bei 1,5 Volt überhaupt keinen, bei 4,5 Volt hingegen nur kaum feststellbare Mengen Sauerstoff freilegte.

An der Platinanode andererseits wurden keine Ionen in den Agar abgegeben, hingegen Sauerstoff in deutlich feststellbarem Ausmass.

Die Meidung der Platinanode könnte vielleicht auf die O_2-Wirkung zurückgeführt werden; wir hatten bereits in den früheren Versuchen mit Sauerstoff eine gewisse Abstossung festgestellt. Es ist jedoch auch denkbar, dass die Anode an sich abstossend wirkt, dass aber im Falle der Kupferanode die in den Agar abgegebenen Kupferionen eine Anziehung bewirken, welche die Abstossung übertrifft. Häufig wurde zum Beispiel die blaue Zone im Agar noch nach Abbruch des Versuches von den Nematoden in grösserem Umfang aufgesucht.

Im Gegensatz zu den Anoden verhielten sich die Kathoden im Agar gleich. Auch ihr Einfluss auf die Nematoden war einheitlicher. Sie wurden im allgemeinen nicht gemieden, in gewissen Versuchen hingegen deutlich bevorzugt.

Jones (1960) fand für *D. dipsaci* in elektrischen Versuchen eine Anziehung zur Kathode. Unsere Experimente zeigen keinen so deutlichen Zusammenhang auf. In Bezug auf das Verhalten gegenüber elektrischem Strom sind aber die Paramaecien vielfach untersucht worden, (siehe z.B. Viaud & Bonaventure, 1956; Dryl 1958). Im allgemeinen wandern diese zur Kathode, sie können aber anodisch werden, wenn sie vorher in gewisse Salze eingetaucht werden. Andere Salze unterdrücken überhaupt jegliche Galvanotaxis. Möglicherweise bewirken die in Lösung

gehenden Kupferionen eine ähnliche Umkehr der Reaktion bei *D. dipsaci,* indem sie ähnlich wirken wie jene Salze. Eventuell entstehen auch günstigere osmotische Bedingungen, welche bevorzugt aufgesucht werden.

Abschliessend möchten wir feststellen, dass das Verhalten von Nematoden gegenüber elektrischem Strom weitere Versuche erfordert. Die Hypothese über die anziehende Wirkung von sinkenden Redoxpotentialgradienten kann jedoch auf Grund der bisherigen elektrischen Experimente mit *D. dipsaci* ebenfalls nicht bestätigt werden.

DISKUSSION

Die von Bird (1959) auf Grund von Experimenten mit *Meloidogyne spp.* aufgestellte Redoxpotential-Hypothese der Anziehung trifft gemäss unseren Versuchsergebnissen für *D. dipsaci* nicht zu.

Übereinstimmung mit den Resultaten des genannten Autors ergab sich immerhin in verschiedenen Punkten, nämlich beim Sauerstoff, der zu keinerlei Ansammlungen Anlass gab, beim pH, das keinen Einfluss auf die Anziehung ausübte und beim Reduktionsmittel Natriumdithionit, welches eine deutlich anziehende Wirkung entfaltete. Beim CO_2 erzielten wir kräftige Anziehung, was unsere früheren Beobachtungen bestätigte. Hingegen erzielte Bird (1959) keine Anziehung, korrigierte diesen Befund aber 1960 (siehe Nachtrag p. 82).

Aus verschiedenen Beobachtungen erklärt sich, warum die Anziehung von *D. dipsaci* nicht auf ein erniedrigtes Redoxpotential zurückgeführt werden kann. Mit dem Reduktionsmittel Natriumdithionit lösten sich die Ansammlungen wieder auf, bevor der fallende ORP-Gradient verschwunden war. Das Oxydationsmittel $KMnO_4$ erhöhte das Redoxpotential und ergab trotzdem Anziehung, während eine Erniedrigung dieses Potentials mit Wasserstoff zu keinem positiven Ergebnis führte. Die sehr deutliche Anziehung in den Versuchen mit Kohlendioxyd wurden bei konstant bleibendem ORP und pH erzielt. Auch in den elektrischen Versuchen kann keine Bestätigung für die Redoxpotentialhypothese gesehen werden.

Es darf angenommen werden, dass eine Anziehung durch verschiedene Faktoren ausgelöst werden kann. Unter diesen dürften die chemischen Eigenschaften der Stoffe eine wichtige Rolle spielen. Möglicherweise sind auch osmotische Bedingungen von einigem Einfluss. Die Wirkung von elektrischem Strom dürfte hingegen hauptsächlich theoretische Bedeutung haben. Unter Berücksichtigung der bisherigen Experimente kann man sich die Auffindung der Wirtspflanzen folgendermassen vorstellen: Die primäre Anziehung über einige Distanz wird durch die CO_2-Abgabe der Wurzeln ermöglicht. In unmittelbarer Wurzelumgebung muss dann mit einer weiteren zusätzlichen Orientierung dank gelöster Ausscheidungen (z.B. Glutaminsäure, Bird, 1959) gerechnet werden, unter denen sich auch reduzierende und oxydierende Stoffe befinden (Lyon & Wilson, 1921; Katznelson et al., 1955).

Das CO_2 übernimmt damit die gleiche Rolle wie bei den bodenbewohnenden Insekten. Die Tatsache, dass das Kohlendioxyd bei Nematoden auch anderweitig

physiologisch wirksam ist, zeigt die Mitteilung von Rogers (1958), wonach dieses Gas bei niedrigem Redoxpotential die Schlüpfrate von *Ascaris lumbricoides* erhöht.

Nachtrag:

Unmittelbar nach der endgültigen Niederschrift des Manuskriptes erreichte uns eine kurze Mitteilung von Bird (1960). Die von Klingler (1959) berichtete Anziehung von *D. dipsaci* zu CO_2-Quellen konnte vom genannten Autor nun an andern Arten bestätigt werden, nachdem er unsere Technik der CO_2-Einleitung übernommen hatte. Er misst der Erniedrigung des ORP für die Anziehung nicht mehr eine so wichtige Rolle bei, wie in seiner 1959 publizierten Arbeit. Immerhin wird im Prinzip an der Hypothese festgehalten.

Die Vorstellungen Bird's und unsere eigenen über die Anziehung haben sich nach dieser Berichtigung angenähert, wobei aber eine Diskrepanz betreffend die Wirkung des erniedrigten Redoxpotentials weiterbesteht.

Bird (1960) äussert seinerseits gewisse Zweifel über die Rolle des Kohlendioxyds zur Erklärung der Ansammlungen in gewissen Wurzelzonen: "However, localisation of nematodes in the region of cell elongation does not appear to be influenced by carbon dioxide as the redox potential is lowest here. If carbon dioxide were present in greater concentrations in this region of the root, it would tend to raise the redox potential". Nun ist aber nach Wanner (1944) der Sauerstoffverbrauch in der Teilungs- und Streckungszone der Wurzel am grössten. Da deren Respirationsquotient CO_2/O_2 unter aeroben Bedingungen gleich 1 ist (Lundegårdh, 1940), müssen die Zonen des grössten O_2-Verbrauchs auch jene der grössten CO_2-Bildung sein. Eine Ansammlung der Nematoden in der Teilungs- und Streckungszone kann also auf Grund der CO_2-Abgabe angenommen werden. Die Aussage, dass das Kohlendioxyd eine Erhöhung des ORP herbeiführe, muss wohl auf einem Missverständnis beruhen. In ORP-Messungen kann anstelle von N_2 notfalls CO_2 treten, um den Einfluss des Luftsauerstoffes auszuschalten (Müller-Reuther, 1953), was heisst, dass — bei konstantem pH — das CO_2-Gas an sich das Redoxpotential nicht beeinflusst. Dies zeigten im übrigen auch unsere Messungen (p. 74). In Bezug auf die Wurzel wurde jedoch schon in der Einleitung betont, dass *Erhöhung* der Stoffwechselaktivität bezw. CO_2-Abgabe und *Erniedrigung* des Potentials gekoppelt sind [1]).

Es bleibt mir abschliessend meinen Kollegen Dr. W. Vogel und Dr. E. Stutz für ihre wertvollen Auskünfte und Herrn Dr. F. Schneider für seine stetige Anteilnahme an dieser Arbeit herzlich zu danken.

[1]) Nach Einreichen meines Manuskriptes wurde ich auf eine Arbeit von Rohde aufmerksam gemacht, die ich übersehen hatte. [ROHDE, R. A. (1960). The influence of carbon dioxide on respiration of certain plant-parasitic nematodes. *Proc. helm. Soc. Wash.* **27**, 160-164]. Es wurde festgestellt, dass die pflanzenparasitären Nematoden in normaler Luft (0,03% CO_2) stärker atmen als in Luft von 1% bezw. 2% CO_2-Gehalt, und in diesen beiden Milieus stärker als in CO_2-freier Luft. Die stärkste Atmung muss also irgendwo zwischen 0% und 1% CO_2-Gehalt auftreten. Darunter und darüber tritt eine Reduktion ein. Parallel zur Reduktion der Atmung wurde in CO_2-freier Luft

SUMMARY

Experiments on the attraction of Ditylenchus dipsaci: *the effect of carbon dioxide, redox potentials and other factors*

Experiments with *Ditylenchus dipsaci* in agar suspension were made to test the effects of carbon dioxide, redox potentials and other factors. Carbon dioxide, hydrogen and oxygen were passed into the agar through a fine cannula at from 0.5 to 12 ml/h. Attraction by carbon dioxide was confirmed. Both oxygen and hydrogen were without effect, except for some evidence of slight repulsion immediately around the opening of the cannula. The reducing agent, sodium dithionite, like hydrogen gas lowered the redox potential but, whereas the latter had no effect, sodium dithionite was attractive. The oxidizing agent, potassium permanganate, raised the redox potential but was also attractive. Attraction to a carbon dioxide source took place in the absence of redox potential and pH gradients, a situation achieved by buffering. No constant attraction to the anode or cathode was detected when an electric current was applied to the agar through simple platinum or copper wire electrodes.

These results are not in accordance with Bird's hypothesis that attraction to roots is caused by lowered redox potentials. Carbon dioxide given off by roots may be responsible for host findings, for soil inhabiting insects it has been shown to act over considerable distances. However, near the roots supplementary and perhaps more specific orientation may be caused by other root exudates.

LITERATUR

Baunacke, W. (1922). Untersuchungen zur Biologie und Bekämpfung des Rübennematoden *Heterodera schachtii* Schmidt. *Arb. Biol. Anst. Land- & Forstw.* **11**, 185-288.

Bird, A. F. (1959). The attractiveness of roots to the plant parasitic nematodes *Meloidygyne javanica* & *M. hapla*. *Nematologica* **4**, 322-335.

—— (1960). Additional notes on the attractiveness of roots to plant parasitic nematodes. *Nematologica* **5**, 217.

Dickinson, S. (1959). The behaviour of larvae of *Heterodera schachtii* on nitrocellulose membranes. *Nematologica* **4**, 60-66.

Dryl, S. (1958). Photographic Registration of Movement of Protozoa. *Bull. Acad. Pol. Sci.* **6**, 429-430.

Gadd, C. H. & Loos, C. A. (1941). Host specialization of *Anguillulina pratensis* (De Man), I. Attractiveness of roots. *Ann. appl. Biol.* **28**, 372-381.

Jones, F. G. W. (1960). Observations and reflections on host finding in plant parasitic nematodes. *Med. LandbHogesch. Gent* **25**, 1009-1024. (Seen in abstract only).

Katznelson, H., Rouatt, J. W. & Payne, T. M. B. (1955). The liberation of Amino acids and reducing compounds by Plant roots. *Plant & Soil* **7**, 35-48.

Klingler, J. (1957). Über die Bedeutung des Kohlendioxyds für die Orientierung der Larven von *Otiorrhynchus sulcatus* F., *Melolontha* & *Agriotes* (Col.) im Boden. *Mitt. Schweiz. ent. Ges.* **30**, 317-322.

—— (1958). Die Bedeutung der Kohlendioxyd-Ausscheidung der Wurzeln für die Orientierung der Larven von *Otiorrhynchus sulcatus* F. und anderer bodenbewohnender phytophager Insektenarten. *Mitt. Schweiz. ent. Ges.* **31**, 205-269.

—— (1959). Anziehung von Collembolen und Nematoden durch Kohlendioxyd-Quellen. *Mitt. Schweiz. ent. Ges.* **32**, 311-316.

Kühn, H. (1959). Zum Problem der Wirtsfindung phytopathogener Nematoden. *Nematologica* **4**, 165-171.

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eine Abnahme der Aktivität beobachtet. Es wird die Hypothese aufgestellt, dass die Reduktion der Atmung bezw. der Aktivität durch höhere CO_2-Konzentrationen zu einer orthokinetisch bedingten Ansammlung bei atmenden Wurzeln führen könnte. Den Nematoden würde durch Abnahme der Lokomotions-Geschwindigkeit das Verlassen der Wurzelzone erschwert. Gegen diesen rein passiven, orthokinetischen Erklärungsversuch der Ansammlung bei CO_2-Quellen spricht allerdings die von uns beobachtete, aktive Einwanderung in die Mündung der CO_2-führenden Kapillaren (Klingler, 1959).

LINFORD, M. B. (1939). Attractiveness of roots and excised shoot tissues to certain nematodes. *Proc. helm. Soc. Wash.* **6**, 11-18.

LUNDEGÅRDH, H. (1940). Investigations as to the absorption and accumulation of inorganic ions. *LantbrHogsk. Ann.* **8**, 233-404.

—— (1942). Electrochemical relations between the root system and the soil. *Soil Science* **54**, 177-189.

LYON, T. L. & WILSON, J. K. (1921). Liberation of organic matter by roots of growing plants. *Mem. Cornell Agr. Exp. Sta.* **40**, 1-44. (Zit. nach Katznelson et al., 1955).

MÜLLER, E. & REUTHER, H. (1953). *Elektrochemisches Praktikum.* Dresden & Leipzig, Theodor Steinkopff, 384 pp.

ROGERS, W. P. (1958). Physiology of the Hatching of Eggs of *Ascaris lumbricoides*. *Nature Lond.* **181**, 1410-1411.

ROVIRA, A. D. (1956). Plant root excretions in relation to the rhizosphere effect. I. The nature of root exudate from oats & peas. *Plant & Soil* **7**, 178-194.

—— (1959). Root excretions in relation to the rhizophere effect. Influence of plant species, age of plant, light, temperature and calcium nutrition on exudation. *Plant & Soil* **11**, 53-64.

SHEPHERD, Audrey M. (1959). The invasion and development of some species of *Heterodera* in plants of different host status. *Nematologica* **4**, 253-267.

THORPE, W. H., CROMBIE, A. C., HILL, R. & DARRAH, J. H. (1947). The behaviour of wireworms in response to chemical stimulation. *J. exp. Biol.* **23**, 234-266.

VIAUD, G. & BONAVENTURE, N. (1956). Recherches expérimentales sur le galvanotropisme des Paramécies. *Bull. biol. France et Belgique* **90**, 287-319.

WALLACE, H. R. (1958). Observations on the emergence from cysts and the orientation of larvae of three species of the genus *Heterodera* in the presence of host plant roots. *Nematologica* **3**, 236-243.

WANNER, H. (1944). Unterschiede in der Atmungsintensität verschiedener Wurzelzonen. *Vierteljahresschr. naturf. Ges. Zürich* **89**, 285-287.

WEISCHER, B. (1959). Experimentelle Untersuchungen über die Wanderung von Nematoden. *Nematologica* **4**, 172-186.

WIESER, W. (1955). The Attractiveness of Plants to Larvae of Root-Knot Nematodes. I The Effect of Tomato Seedlings and excised Roots on *Meloidogyne hapla* Chitwood. *Proc. helm. Soc. Wash.* **22**, 106-112.

—— (1956). The Attractiveness of Plants to Larvae of Root-Knot Nematodes. II The Effect of excised Bean, Eggplant and Soybean Roots on *Meloidogyne hapla* Chitwood. *Proc. helm. Soc. Wash.* **23**, 59-64.

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Nematologica 6 (1961): 85-86. Leiden, E. J. Brill

SHORT COMMUNICATION

F. C. Peacock [1]): *A note on the attractiveness of roots to plant parasitic nematodes.*

Kühn (1959) suggested that nematodes wander at random, meet host roots by chance and enter or alter movements only then under the influence of substances emitted by the roots. Wallace (1960) discussed this possibility but favoured the theory of orientation towards an attractive chemical root exudate. Peacock (1959) qualitatively and Lownsbery & Viglierchio (1958, 1960) quantitatively demonstrated attraction of nematodes to roots which they were prevented from reaching by a dialytic membrane, and interpreted their results as evidence for a dialisable chemical attractant emanating from the root.

Attraction to carbon dioxide released by respiring roots was proposed by Klingler (1959) and discussed by Bird (1960) who, however, showed that a piece of living root exerted stronger attraction than did carbon dioxide diffusing through the tip of a capillary. Rhode (1960) suggested that carbon dioxide released from respiring roots decreased nematode activity and thus prevented them from leaving the root area. This seems unlikely in view of the evidence for positive attraction and the fact that greater activity would be required for penetration of the root (which occurs in a presumably high CO_2 area) than would be needed merely to leave the area. Bird (1959) showed that certain reducing agents attracted nematodes, and proposed a theory of attraction to areas of low redox potential. In a later communication, however, (Bird 1960) he attached less importance to these factors and concluded that root exudation played a greater part. The nature of the attractant substance(s) in these exudations remains to be demonstrated.

The extent of the dependence of root-knot nematodes on the attractant substance for finding and penetrating the host root is shown in the following simple experiments in which activated charcoal and ion exchange resins were used to remove attractant substances from the medium.

METHOD

Tomato seedlings were pricked out into mixtures of either vermiculite or sand with finely-divided charcoal. After 7 days 300 24-hour old larvae of *Meloidogyne incognita* were pipetted into each pot. At intervals plants were washed free of the mixture and the number of galls counted.

RESULTS

Results in Table I are representative of those obtained in a number of similar experiments.

Table I

Effect of activated charcoal on invasion of tomato roots by Meloidogyne *larvae*

	% charcoal by wt.	No. of galls per plant after 10 days	after 17 days	after 38 days
Vermiculite alone	—	148	102	102
„ and charcoal	6.3	7	10	9
„ „ „	12.5	7	4	11
„ „ „	25	0	0	1
„ „ „	50	0	0	0
				after 45 days
Sand alone	—		65	49
„ and charcoal	0.8		2	5
„ „ „	1.6		0	7
„ „ „	3.1		2	6
„ „ „	6.3		0	4
„ „ „	12.5		0	2

1) Imperial Chemical Industries Limited Jealott's Hill Research Station, Bracknell, England.

These results, however, may be explained by proposing the rapid removal from the system either of attractant carbon dioxide or of attractant exudate, so the experiment was repeated with a range of ion exchange resins compared with charcoal and including cellulose and bentonite (Table II). Only charcoal prevented nematode invasion of the roots: had carbon dioxide been the attracting factor one would have expected the basic anion exchange resins to have given a similar result.

TABLE II

Effect of adsorbent materials and ion-exchange resins on invasion of tomato roots by Meloidogyne *larvae*

	% by wt.	Root Grading 10 Marks for best plant	No. of galls per plant after 13 days	after 40 days
Control. Sand alone	—	7	19	61
Sand + Cellulose	2.0	9	67	176
	4.0	7	88	107
„ + Charcoal	2.0	9	1	4
	4.0	10	0	2
„ + Bentonite	2.0	9	82	174
	4.0	8	96	189
„ + Zeocarb 225	2.0	7	53	107
strongly acidic	4.0	6	35	84
„ + Deacidite FF	2.0	10	35	107
strongly basic	4.0	8	23	126
„ + Deacidite G	2.0	10	23	167
weakly basic	4.0	10	21	127
„ + Deacidite H	2.0	10	81	172
„	4.0	9	37	177

Zeocarbs 215, 216 and 226 (acidic) and De-Acidite E (basic) were also tested but were markedly phytotoxic and so have not been included in this Table.

Miss Jean Taylor and Mr. J. Clayton helped with these experiments. Dr. A. Calderbank confirmed experimentally that the basic resins used did, in fact, effectively remove CO_2 from a moist sand environment.

REFERENCES

BIRD, A. F. (1959). The attractiveness of roots to the plant parasitic nematodes *Meloidogyne javanica* and *M. hapla*. *Nematologica*, **4**, 322-335.

—— (1960). Additional notes on the attractiveness of roots to plant parasitic nematodes. *Nematologica*, **5**, 217.

KLINGLER, J. (1959). Anziehung von Kollembolen und Nematoden durch Kohlendioxyd-Quellen. *Mitt Schweiz. ent. Ges.* **32**, 311-316.

KÜHN, H. (1959). Zum Problem der Wirtsfindung phytopathogener Nematoden. *Nematologica*, **4**, 165-171.

LOWNSBERY, B. F. & VIGLIERCHIO, D. R. (1958). Mechanism of accumulation of *Meloidogyne hapla* around roots of tomato seedlings. *Phytopathology*, **48**, 395.

—— & —— (1960). Mechanism of accumulation of *Meloidogyne incognita acrita* around tomato seedlings. *Phytopathology*, **50**, 178.

PEACOCK, F. C. (1959). The development of a technique for studying the host/parasite relationship of the root-knot nematode *Meloidogyne incognita* under controlled conditions. *Nematologica*, **4**, 43-55.

RHODE, R. A. (1960). The influence of carbon dioxide on respiration of certain plant-parasitic nematodes. *Proc. helm. Soc. Wash.* **27**, 160-164.

WALLACE, H. R. (1960). Movement of eelworms. VI. The influence of soil type, moisture gradients and host plant roots on the migration of the potato root eelworm *Heterodera rostochiensis* Wollenweber. *Ann. appl. Biol.* **48**, 107-120.

BOOK REVIEW

J. N. Sasser and W. R. Jenkins. *Nematology, Fundamentals and Recent Advances, with emphasis on Plant Parasitic and Soil Forms.* University of North Carolina Press, Box 510, Chapel Hill, N.C., USA, 1960. 162 figures, 79 tables, XV + 480 pages. Price: $ 12.50.

Suitable texts for teaching and general reference purposes have been notably lacking in nematology and this publication is most welcome as a highly valuable and encouraging contribution to this need. There is yet to be published an up-to-date textbook comprehensive enough for introductory class purposes and as a reference for the layman. But the compilation reviewed here should be especially useful to the graduate student and the professional nematologist.

It is noteworthy for the authoritative treatment with extensive bibliographical lists given to a wide range of the fundamentals of nematology. Generous use is made of literature on all aspects including parasitology, marine and free-living forms as well as phytonematology.

This book serves the further purpose of drawing together many separate disciplines which contribute to nematology. The fragmentation in past years of nematological research and teaching into many separate fields such as helminthology, parasitology, plant pathology, entomology, invertebrate zoology, etc., has been a deterrent to the science of nematology. Unified efforts such as this should be of inestimable value in emphasizing the common interests and basic relationships of all nematological research. Future work can only be richer, more effective and productive with a more intimate association and a freer exchange of ideas and information.

The source of this compilation was a series of lectures and laboratory exercises presented during a Southern Regional Summer Session in Nematology at North Carolina State College, June 8-July 17, 1959. Twenty-two scientists from Canada, England, The Netherlands, Puerto Rico and the United States participated. Included is a general plan of organization for a nematology program and a comprehensive review of methods, equipment and techniques used in nematology. Special attention is given to radio-isotopes and the Cartesian diver apparatus, their use and potential application in nematological research.

The gross morphology, internal and external anatomy of nematodes are covered including an extended series of illustrations. Special taxonomic-morphologic treatment is given to selected groups of nematodes with keys to certain of the genera.

The physiology and biochemistry of nematodes is especially well done with attention to factors influencing metabolic rate of nematodes, the influence of oxygen, pH, ions, osmotic pressure, temperature, etc., on their life processes. Methods for the cultivation of Aschelminths, especially rhabditid nematodes, presented in detail, will be useful in nutritional, biochemical genetics, growth and differentiation studies.

The genetics and oogenesis of nematodes are reviewed and the possible use of chromosome cytology in taxonomy is discussed. There is an especially comprehensive review of the literature on nematode ecology. The section on host-parasite relations covers an analysis of possible plant-nematode relationships, proof of pathogenicity, mechanisms involved in plant-nematode relationships, interrelationships between nematodes and other soil-borne pathogens.

The control of plant parasitic nematodes of special concern in agricultural production is discussed in all aspects with the exception of the lectures on the Theory of Soil Fumigation which have been published already as a separate paper by the Dow Chemical Company.

D. J. Raski

NACHRUF

C. A. ALLGÉN ZUM GEDENKEN

Von I. Andrássy

Am 19. August 1960 verstarb Dr. C. A. Allgén 74 Jahre alt in Malmö (Schweden), wo er die letzten Jahre seines Lebens verbrachte. C. A. Allgén wurde am 21. Juli 1886 in Lund geboren. Dort beendete er auch seine Universitätsstudien. Seine wissenschaftliche Tätigkeit begann er zunächst als Botaniker und wandte sich erst im 37. Lebensjahr den Nematoden zu. Aus seiner Feder sind mehr als 200 wissenschaftliche Arbeiten hauptsächlich über marine Nematoden hervorgegangen. Daneben veröffentlichte Allgén auch wertvolle Beiträge über binnenländische Arten. Er konnte es noch erleben, dass vor seinem Tode seine fast 300 Seiten umfassende Arbeit „Freeliving marine nematodes" erschien. Neben systematischen, tiergeographischen und ökologischen Untersuchungen widmete sich Allgén auch der Biologie und Physiologie der Nematoden. Mit C. A. Allgén hat die Nematodenforschung einen Menschen von grossem Wissen verloren.

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OBSERVATIONS ON THE REPRODUCTION OF *APHELENCHOIDES RITZEMABOSI* (SCHWARTZ)

BY

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Examination of leaves inoculated with known numbers of eelworms showed the egg stage of *Aphelenchoides ritzemabosi* lasted 4-5 days and the generation time 11-12 days in chrysanthemums grown in the greenhouse and 13-14 days in plants grown outside. Parthenogenesis did not occur and fertilized females survived in dried leaves, stored at 6°C for 6 months and reproduced without refertilisation.

Except for observations by Stewart (1921) working with *Aphelenchoides ritzemabosi* in groundsel (*Senecio vulgaris* L.) the life history of this nematode has received little attention. He concluded that embryonic development took up to 5 days, complete maturation up to 10 days, and that the cycle from egg to egg could be completed in 14 days if the day temperature did not fall below 15°C. He drew these conclusions on finding young larvae 5 days, and older larvae and young adults 10 days, after infecting groundsel leaves.

Reproduction without males has been studied by Maupas (1900) who listed forms which can reproduce in this way. Mulvey (1958) found that parthenogenesis occurs in *Heterodera trifolii* Goffart and Tyler (1933) that a species of *Meloidogyne* which reproduces sexually, can reproduce without males. Ellenby (1957) and Fassuliotis (1957) reported that parthenogenesis did not occur in *H. rostochiensis* Wollenweber and Golden (1959) that it did not occur in *H. schachtii* Schmidt. Males of *A. ritzemabosi* are generally found in association with females but, as far as is known, the possibility of parthenogenesis in leaf and bud eelworms has not been studied.

Reproduction of overwintering females has likewise received little attention. This species can survive at least 2 years in a dormant state (Steiner (1924), Voss (1930), Goodey (1933)) but whether a fertilised female, after overwintering, can enter a new host and reproduce without refertilisation was unknown.

The present work was designed to give further information on the life history, to see if parthenogenesis occurred and to study the infestations developing from overwintering females, using chrysanthemums, the most commonly encountered host.

LIFE HISTORY

Method

Eelworms were extracted from the current season's (1960) chrysanthemum leaves by Baermann funnel and added to drops of water on the underside of six to eight leaves on 48, 6 week old chrysanthemum plants known to be free from infection. Single, mature females were placed on half the leaves and ten mature females on each of the remaining leaves. Each plant, after inoculation, was covered with a polythene bag for 2 days to reduce evaporation and give the nematodes time to enter the leaves. After removing the bags, 32 plants were left in the greenhouse and 16 placed outside under a polythene roof to ensure the leaves were kept dry, so minimising the risk of movement of eelworms from leaf to leaf.

Eggs, larvae and adults were counted after staining with acid fuchsin-lactophenol. Samples of leaves were taken daily from the greenhouse plants and every 2-3 days from the outdoor plants. After 14 days, when nematode numbers became too great to count by this method, they were extracted by Baermann funnel and counted in a marked Syracuse watch glass. Although the size of the sample varied it was never less than three leaves per treatment at each examination.

Results

Staining showed that females in the inoculated leaves were generally well distributed and moved very little after entering the leaf. It was always possible to associate them with eggs and larvae they had produced. Under greenhouse conditions, with mean daily temperatures between 17° C and 23° C, eggs appeared by the second and larvae by the seventh day after inoculation (Fig. 1).

Mature females were present on the thirteenth day and thereafter there was an apparently uniform increase in larvae and adults (Fig. 2), the eggs becoming too numerous to count. In the greenhouse the egg stage lasted 4-5 days, the larvae developed to mature adults in 7-8 days and the generation time from egg to egg was 11-12 days. Outside, where the daily mean temperatures ranged between 13° C and 18° C, the generation time was 13-14 days. Because larvae were hatching at a uniform rate and because the number of eggs after the sixth day remained constant at approximately ten per female (Fig. 1), eggs were being produced at the rate of about two per day. This conclusion is supported by the average number of nematodes produced per female during the generation: 22 on the greenhouse plants and 23 on those growing outside. A maximum of 36 was encountered in one leaf which had been inoculated with a single female.

Although the mean number of adults and larvae showed a steady increase (Fig. 2), the variations in numbers from leaf to leaf was considerable. For example, the numbers in leaves inoculated with ten females were on the 13th day 26, 78, 97, 145, on the 29th day 313, 620, 968 and on the 31st 100, 312, 624, 750 and 1,750. The reasons for such variations are not certain but indifferent growing conditions and the effect of the eelworm population itself may be important factors. The maximum number recovered from a leaf inoculated with a single female was 272

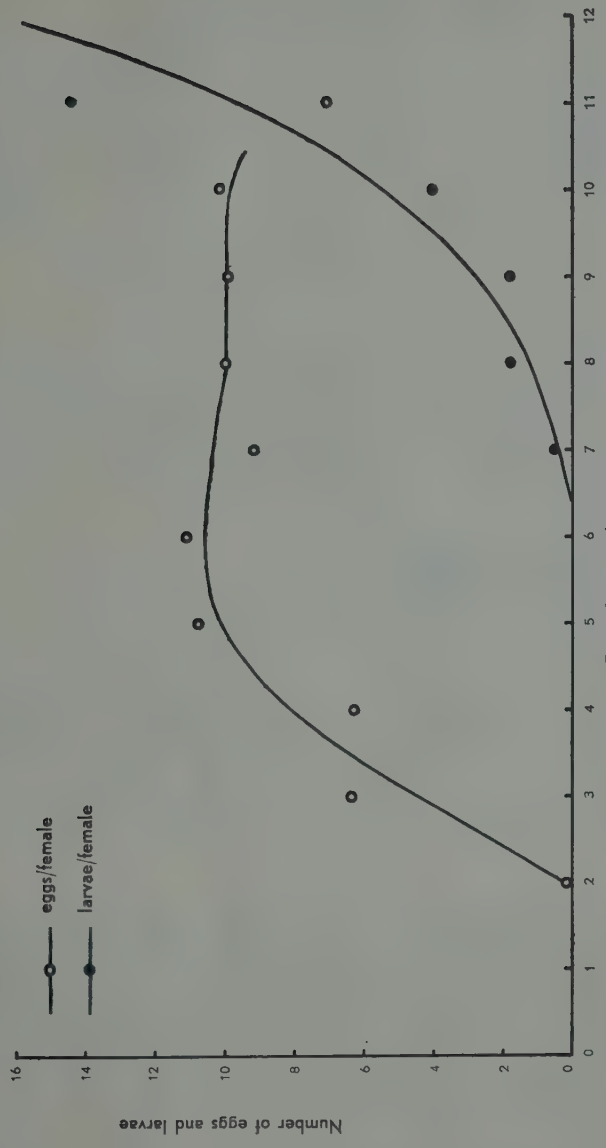

Fig. 1. Mean number of eggs and larvae in chrysanthemum leaves inoculated with 10 females.

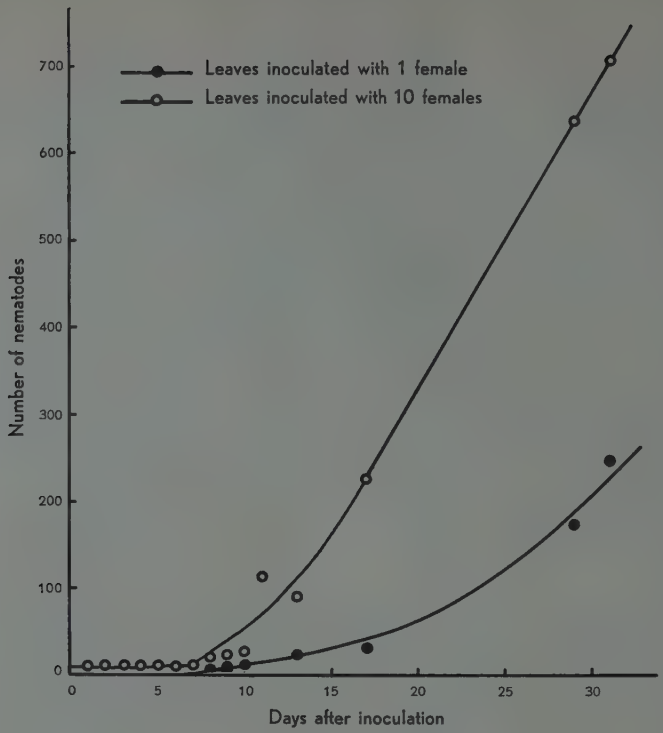

Fig. 2. Mean number of adults and larvae per leaf.

after 29 days, 724 after 31, and 3,500 after 38 days, the end of three generations. The sex ratio (males : females) varied from 1 : 3 to 1 : 8, the mean ratio being 1 : 4.6.

PARTHENOGENETIC REPRODUCTION

Method

Single larvae, dissected under water from dried leaves stored at 6° C from November 1959 to May 1960, were placed on individual, eelworm free, chrysanthemum leaves. Sixty leaves were inoculated. In addition two groups of eighteen leaves were similarly inoculated with larvae from unstored, fresh material but in one group five males were added to each leaf. Leaves were examined by staining or by Baermann funnel extraction for a period of 12 weeks.

Results

The 60 leaves inoculated with larvae from stored leaves yielded eleven females

and four males, i.e., only 25 % of the larvae developed to maturity. In no case did the females produce eggs. Of the nematodes recovered fourteen were found within 6 weeks of inoculation, the remaining one being found in the 13th week. The low recovery may have been due either to storage having affected the vitality of the larvae or to mortality before the leaves were examined.

From the leaves inoculated with eelworms from unstored leaves, five females, none of which had reproduced, were recovered from the group inoculated with larvae only, while reproduction had occurred in ten of the eighteen leaves inoculated with a larva and five males. These results indicate that males are necessary for reproduction.

REPRODUCTION BY OVERWINTERING NEMATODES

Method

Females dissected under water from dried leaves stored for 6 months (November-May) at 6° C, were immediately transferred to the underside of chrysanthemum leaves. Seventy five leaves were inoculated with a single female and 75 with a male and a female. To determine whether reproduction had taken place, four leaves from each group were examined very 4-5 days by staining of Baermann funnel extraction.

Results

Nematodes were recovered from 68 % of the leaves inoculated with a male and a female, reproduction having occurred in 65 %, compared with 64 % recovery from leaves inoculated with a single female, reproduction having occurred in 63 %. After 54 days the highest number extracted from a leaf inoculated with a male and female was 5,200 compared with 7,500 from a leaf inoculated with a female only. Clearly a single fertilised female can survive the winter in the dry state, infect a leaf and reproduce without refertilisation, the presence of a male having no effect on the reproductive capacity.

DISCUSSION

The duration of the egg stage (4-5 days) and the generation time (11-14 days) agree closely with the conclusions of Stewart (1921). During the first generation, each female produced an average of 22 offspring. But this rate of reproduction was not maintained. Many of the infested leaves died and, because the plant surface was kept dry to prevent migration from leaf to leaf, shortage of food supply is a possible explanation of the fall in reproductive rate. If reproduction continued at this rate nearly 2,000,000 eelworms would be produced in five generations, completed in 2 months. Normally the population would never increase at such a rate because of environmental factors but the high reproductive potential may account for the extremely rapid build-up when conditions are favourable. The speed of increase emphasises the need for efficient control measures.

Since parthenogenetic reproduction does not occur, it is of biological significance that a fertilised female can survive extended periods in dried leaves and reproduce without refertilisation.

We thank Mr. J. H. White and Dr. W. H. Golightly for helpful criticism of this paper.

ZUSAMMENFASSUNG

Bemerkungen über die Vermehrung von Aphelenchoides ritzemabosi *(Schwartz)*

Zum Studium von Lebensgeschichte und Vermehrungsmass von *Aphelenchoides ritzemabosi* wurden Chrysanthemumblätter mit einer bestimmten Anzahl Weibchen infiziert. Eier, Larven und erwachsene Tiere wurden nach Färbung infizierter Blätter in den ersten 14 Tagen jeden Tag gezählt, später nach Extraktion im Baermann Trichter. Das Eistadium dauerte 4-5 Tage, die Generationszeit 11-12 Tage bei Pflanzen im Gewächshaus, 13-14 Tage bei Pflanzen im Freien. Jedes Weibchen produziert während der ersten Generation im Durchschnitt 22 Nachkommen. Etwa 2 Eier werden je Tag abgelegt. Diese Vermehrungsrate wurde nicht beibehalten. Nach 38 Tagen betrug die höchste Zahl, die aus einem mit einem einzelnen Weibchen infizierten Blatt extrahiert wurde, 3500. Das durchschnittliche Geschlechtsverhältnis betrug 1 Männchen zu 4,6 Weibchen.

Chrysanthemumblätter wurden mit einzelnen Larven allein und mit einzelnen Larven plus fünf Männchen infiziert, um festzustellen, ob eine parthenogenetische Vermehrung möglich ist. Andere Blätter wurden mit einzelnen Weibchen infiziert, die aus trockenen über Winter aufgehobenen Blätter extrahiert wurden. Parthenogenese trat nicht auf. Befruchtete Weibchen überlebten in trockenen bei 6° C für 6 Monate aufgehobenen Blättern und vermehrten sich ohne erneute Befruchtung.

REFERENCES

Ellenby, C. (1957). An investigation into the possibility of parthenogenesis in the potato-root eelworm, *Heterodera rostochiensis* Wollenweber. *Nematologica*, **2**, 250-254.

Fassuliotis, G. (1957). Role of the male in reproduction of the golden nematode. *Phytopathology*, **47**, 11.

Golden, A. M. (1959). Significance of males in reproduction of the sugar-beet nematode (*Heterodera schachtii*). *Plant Dis. Reptr* **43**, 979-980.

Goodey, T. (1933). *Plant parasitic nematodes and the diseases they cause*. London, Methuen, 306 pp.

Maupas, E. (1900). Modes et formes de réproduction des nématodes. *Arch. Zool. exp. gén. sér.* 3. **8**, 463-624.

Mulvey, R. H. (1958). Parthenogenesis in a cyst-forming nematode, *Heterodera trifolii* (Nematoda: Heteroderidae). *Canad. J. Zool.* **36**, 91-93.

Steiner, G. (1924). On some plant parasitic nemas and related forms. *J. agric. Res.* **28**, 1056-66.

Stewart, F. H. (1921). The anatomy and biology of the Parasitic Aphelenchi. *Parasitology*, **13**, 160-179.

Tyler, J. (1933). Reproduction without males in aseptic root cultures of the root-knot nematode. *Hilgardia*, **7**, 373-388.

Voss, W. (1930). Beiträge zur Kenntnis der Älchenkrankheit der Chrysanthemum. *Z. Parasitenk.* **2**, 310-356.

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NOTE PRÉLIMINAIRE SUR LE DÉPLACEMENT DE *HEMICYCLIOPHORA PARADOXA* LUC (NEMATODA — CRICONEMATIDAE) DANS LE SOL

PAR

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L'auteur étudie le déplacement vertical de *Hemicycliophora paradoxa* hors d'une terre infestée venant de culture de mil (*Pennisetum typhoides*) en pot, vers de la terre stérilisée ou vers de la terre de rhizosphère de mil mise en contact dans des tubes de plexiglas. Les résultats des diverses expériences montrent que le déplacement vers la terre stérilisée est faible et est augmenté par un courant ascendant d'humidification (H = 5 - 20 %). Si l'on remplace la terre stérilisée par de la terre de rhizosphère de mil les taux de déplacement montent encore et avec humidification deviennent les plus hauts. La culture du mil, plante-hôte, a donc apporté au sol un ou des facteurs favorisant les déplacements de *H. paradoxa*. D'autres séries expérimentales ont montré que ce ou ces facteurs sont adsorbés sur la fraction argileuse du sol, sont thermolabiles, résistants à la dessication, rémanents dans le sol après la culture du mil et diffusent à des distances assez grandes de son système radiculaire aussi bien horizontalement que verticalement. D'autre part, la culture de différentes Légumineuses, plantes non hôtes, ne semble apporter au sol aucun facteur favorisant. Enfin la culture du mil apporterait au sol, en sus du ou des facteurs favorisants, un ou des facteurs hydrosolubles, défavorisant le déplacement.

Le but de cette note est de faire part de quelques résultats obtenus lors de recherches, encore en cours, sur certains aspects du déplacement des nématodes phytoparasites dans le sol et les facteurs qui les gouvernent.

Hemicycliophora paradoxa Luc fut choisi comme animal d'expérience pour plusieurs raisons: sa facilité de reproduction sur des graminées cultivables en pot, l'habitus particulier des adultes et des juvéniles permetttant des comptages aisés même en cas de présence accidentelle de saprophages dans l'échantillon, enfin et surtout pour son mode de parasitisme: il s'agit en effet d'un ectoparasite migrateur et de ce fait il est aisé de récupérer, dans le sol, la totalité des populations de cette espèce.

La terre utilisée, légère et sableuse, avait la constitution physique suivante:

argile $<$ 0,002 mm	13,25 %
limon 0,002 $\leqslant$ 0,02 mm	1
sable fin 0,02 $\leqslant$ 0,2 mm	36,7
sable grossier 0,2 $\leqslant$ 2 mm	47,57
gravier $>$ 2 mm	0
matière organique	2,5
	101,02

Toute la terre utilisée était préalablement stérilisée à l'autoclave (120°C pendant 30 mn.). La „terre de mil", dont il sera question plus loin, était obtenue en cultivant du mil (*Pennisetum typhoides* (Burm.) Stapf. & Hubb.) pendant 2 mois, sur cette terre autoclavée, à partir de graines stérilisées superficiellement à l'hypochlorite de calcium. Avant utilisation, cette terre de mil, après arrachage de la plante, était passée au tamis de 10 meshes/inch afin d'enlever tout débris de racine. La „terre infestée" par *H. paradoxa,* était obtenue en introduisant en plusieurs points dans des pots de mil un total de 600 à 1.000 *H. paradoxa,* 10 jours après la sortie des jeunes tiges; après deux mois la population atteignait 15.000 à 35.000 individus au litre de sol et pouvait être utilisée dans les différentes expériences.

Pour étudier le déplacement vertical, le dispositif suivant fut utilisé: un tube de plexiglas (ou „colonne") de 10 cm de diamètre intérieur et de 50 cm de long est pourvu de fentes sur un demi-diamètre, fentes régulièrement espacées tous les cinq centimètres; ces fentes, fermées avec du scotch pendant l'expérience permettent à la fin de celli-ci de diviser la colonne de terre incluse dans le tube en 10 fractions égales, chacune correspondant à 390 cc de terre environ.

La terre infestée par *H. paradoxa* remplissait 3 des fractions de la colonne (15 cm de hauteur), le reste de la colonne (7 fractions: 35 cm) était rempli avec de la terre à tester. Suivant la série d'expériences, la terre infestée était placée soit à la partie supérieure de la colonne et la terre à tester à la partie inférieure, soit à la partie inférieure, soit, enfin, au centre. Dans le premier cas on observait ainsi les déplacements verticaux vers le bas de *H. paradoxa* hors de la terre primitivement infestée dans la terre à tester, dans le second les déplacements verticaux vers le haut.

La population de *H. paradoxa* est extraite séparément de chacune de ces fractions de sol par passage à l'élutriateur de Seinhorst.

La durée des expériences a été de 5 jours et elles ont été effectuées à la température ambiante (27°-33°C).

La valeur de la population de *H. paradoxa* ayant migré hors de la terre originellement infestée dans la terre à tester, ou „taux de déplacement", est exprimée en % de la population totale restant à la fin de l'expérience dans l'ensemble terre infestée + terre à tester.

RÉSULTATS

Déplacements verticaux dans de la terre stérilisée

Dans cette série d'expériences la terre infestée est mise au contact de terre stérilisée, dans les colonnes précédemment décrites. Les deux types de terre ont été préalablement ramenés à la même humidité (H° ± 7 %).

Dans cette série, 3 types d'expériences ont été montés:

Série A-1: la terre infestée est placée à la partie supérieure de la colonne (déplacements vers le bas). Résultats de 3 répétitions:

Série A-1

Population totale de la colonne (a)	Population s'étant déplacée dans la terre stérilisée (b)	Taux de déplacement (b en % de a)
15.815	485	3,0 %
17.010	20	0,1 %
11.085	180	1,6 %
	moyenne	1,5 %

Série A-2: la terre infestée est placée dans le bas de la colonne, sur 15 cm; la terre stérilisée dans le haut (déplacement vers le haut). Résultats de 3 répétitions:

Série A-2

Population totale de la colonne (a)	Population s'étant déplacée dans la terre stérilisée (b)	Taux de déplacement (b en % de a)
9.865	75	0,7 %
23.735	455	1,9 %
11.520	80	0,7 %
	moyenne	1,1 %

Série A-3: la terre infestée est placée au milieu de la colonne sur 15 cm (3 fractions) et de chaque côté est placée de la terre stérilisée sur 15 cm également. Résultats de 4 répétitions:

Série A-3

Population totale de la colonne (a)	Population s'étant déplacée dans la terre stérilisée (b)	Taux de déplacement (b en % de a)
1.645	15	0,9 %
3.775	35	0,9 %
17.460	100	0,5 %
15.055	65	0,4 %
	moyenne	0,6 %

D'après les 3 séries d'expériences on voit que les déplacements verticaux de *H. paradoxa* de la terre de mil infestée vers la terre stérilisée sont faibles, tant vers le haut que vers le bas.

Influence d'un courant ascendant d'humidification

Dans ce type d'expérience, on reprend le même modèle de colonne en plexiglas, mais son extrémité inférieure, fermée par un tamis de 300 meshes inch, est plongée dans un cristallisoir contenant de l'eau de telle façon que celle-ci affleure exactement la surface inférieure de la terre; un dispositif maintient ce niveau constant.

Dans ces conditions la terre contenue dans la colonne s'humidifie progressivement par capillarité; il se crée donc un gradient d'humidité décroissant de bas en haut; l'équilibre est obtenu en 2 jours environ; à ce moment le gradient d'hu-

midité décroît régulièremet, de bas en haut, de 19,1-21 % (subsaturation) à 4,7-5,6 %. L'évaporation dans la partie supérieure de la colonne entretient un léger courant ascendant par capillarité pendant toute la durée de l'expérience. Les trois mêmes modes de disposition de la terre infestée sont repris dans cette série d'expériences:

Série B-1: terre infestée placée à la partie supérieure de la colonne. Le taux de déplacement vers le bas, pour 3 répétitions, est le suivant:

Série B-1

Population totale de la colonne (a)	Population s'étant déplacée dans la terre stérilisée (b)	Taux de déplacement (b en % de a)
18.220	650	3,5 %
13.235	535	4,0 %
16.485	1.525	9,2 %
	moyenne	4,1 %

Série B-2: terre infestée placée dans le bas de la colonne. Le taux de déplacement vers le haut, pour 3 répétitions, est le suivant:

Série B-2

Population totale de la colonne (a)	Population s'étant déplacée dans la terre stérilisée (b)	Taux de déplacement (b en % de a)
7.920	530	6,6 %
15.490	1.300	8,3 %
17.385	4.475	25,7 %!
	moyenne	13,5 %!

Série B-3: terre infestéé placée au milieu de la colonne. Un seul essai à été réalisé, ce type de disposition n'apportant rien de nouveau. Résultat:

Série B-3

Population totale de la colonne (a)	Population s'étant déplacée dans la terre stérilisée (b)	Taux de déplacement (b en % de a)
13.035	930	7,1 %

On constate donc dans ces 3 séries B un déplacement des *H. paradoxa* beaucoup plus important que dans les 3 séries A homologues, sans courant ascendant d'humidification.

De plus, ce courant n'agit pas seulement par entraînement passif des nématodes puisque, dirigé de bas en haut, il favorise le déplacement de haut en bas.

L'influence de l'humidité du sol sur les déplacements de divers nématodes a déjà été étudiée, notamment par Peters (1953) et Wallace (1959, 1960).

Influence d'une plante hôte, le mil (Pennisetum typhoides)

Dans les séries suivantes d'expériences, la terre stérilisée simple a été remplacée par de la terre de mil. Les mêmes types d'expériences sont repris.

Série C-1: terre infestée placée à la partie supérieure de la colonne. Résultats pour 4 répétitions:

Série C-1

Population totale de la colonne (a)	Population s'étant déplacée dans la terre de mil (b)	Taux de déplacement (b en % de a)
27.760	4.990	21.9 %
19.190	1.645	8,5 %
7.705	1.305	16,9 %
13.245	1.445	10,9 %
	moyenne	14,5 %

Série C-2: terre infestée placée à la partie inférieure de la colonne. Résultats pour 3 répétitions:

Série C-2

Population totale de la colonne (a)	Population s'étant déplacée dans la terre de mil (b)	Taux de déplacement (b en % de a)
23.825	1.025	4,3 %
22.730	830	3,6 %
22.930	730	3,1 %
	moyenne	3,6 %

Séries C-3: terre infestée placée au milieu de la colonne. Résultats pour 5 répétitions:

Série C-3

Population totale de la colonne (a)	Population s'étant déplacée dans la terre de mil (b)	Taux de déplacement (b en % de a)
11.260	1.350	11,9 %
16.850	1.340	7,9 %
2.935	395	13,4 %
19.540	1.220	6,2 %
16.855	785	4,6 %
	moyenne	8,8 %

Comparées avec les séries d'expériences homologues réalisées à l'aide de terre stérilisée simple (séries A), on voit que le déplacement est beaucoup plus important dans la terre de mil.

La culture du mil sur de la terre stérilisée a donc rendu le milieu plus favorable au déplacement de *H. paradoxa.*

De plus, ces expériences mettent mieux en évidence le fait que le déplacement vertical vers le bas (séries 1) est plus important que vers le haut (séries 2).

La modification apportée au sol par la culture du mil pouvait être soit physique, soit beaucoup plus probablement, chimique (excrétions radiculaires). Dans ces conditions il pouvait sembler intéressant de voir si un facteur physique à action positive sur le déplacement, en l'occurence un courant ascendant d'humidification, additionnait ou non son action à celle du mil.

Influence simultanée du mil et d'un courant ascendant d'humidification

Le dispositif expérimental est le même que pour la série B, mais la terre stérilisée est remplacée par de la terre de mil.

Série D-1: terre infestée placée à la partie supérieure de la colonne. Résultats pour 4 répétitions:

Série D-1

Population totale de la colonne (a)	Population s'étant déplacée dans la terre de mil (b)		Taux de déplacement (b en % de a)
22.475	4.355		19,3 %
14.540	2.540		17,4 %
5.705	1.540		26,9 %
14.205	2.240		15,7 %
		moyenne	19,8 %

Série D-2: terre infestée placée à la partie inférieure de la colonne. Résultats pour 3 répétitions:

Série D-2

Population totale de la colonne (a)	Population s'étant déplacée dans la terre de mil (b)		Taux de déplacement (b en % de a)
22.155	9.060		40,8 %
14.220	4.570		32,1 %
17.305	5.640		32,5 %
		moyenne	35,1 %

Série D-3: terre infestée placée au milieu de la colonne. Résultats pour 3 répétitions:

Série D-3

Population totale de la colonne (a)	Population s'étant déplacée dans la terre de mil (b)		Taux de déplacement (b en % de a)
10.110	4.110		40,6 %
10.240	3.570		34,8 %
13.510	4.500		33,3 %
		moyenne	36,2 %

Les expériences de 3 séries D montrent, comparées aux séries homologues B et C, que l'influence d'un courant d'humidification ascendant et celle d'une culture de mil s'additionnent.

Le taux du déplacement avec courant ascendant d'humidification, quand on passe de la terre stérilisée à la terre de mil, est multiplié, en moyenne, par 2,6 à 5,1 suivant les séries.

Le système radiculaire du mil a donc modifié le sol et il semble logique de penser que cette modification soit plutôt chimique que physique.

Les résultats pour les 4 séries sont schématisés dans le tableau I:

TABLEAU I

Taux de déplacement dans les séries expérimentales différentes

Terre à tester / Position de la terre infestée	*Séries 1* Terre infestée à la partie supérieure de la colonne	*Séries 2* Terre infestée à la partie inférieure de la colonne	*Séries 3* Terre infestée à la partie médiane de la colonne
Séries A Terre stérilisée	1,5 % * (0,1-3 %) **	1,1 % (0,7-1,9 %)	0,6 % (0,4-0,9 %)
Séries B Terre stérilisée avec courant d'humidification	4,1 % (3,5-9,2 %)	13,5 %! (6,6-25,7 %!)	7,1 % (7,1 %)
Séries C Terre de mil	14,5 % 8,5-21,9 %)	3,6 % (3,1-4,3 %)	8,8 % (4,6-11,9 %)
Séries D Terre de mil avec courant d'humidification	19,8 % (15,7-26,9 %)	35,1 % (32,1-40,8 %)	36,2 % (33,3-40,6 %)

* le premier chiffre représente la moyenne de la série.

** les chiffres entre parenthèses représentent les extrêmes de la série.

Recherches sur le ou les facteurs apportés au sol par le mil et favorisant les déplacements de H. paradoxa

Résistance au lavage

Quatre colonnes furent préparées comme suit:

E1 — Terre infestée entre 2 lots de terre stérilisée (H° ≏ 7 %).

E2 — Terre infestée entre 2 lots de terre de mil (H° ≏ 7%).

E3 — Terre infestée entre 2 lots de terre stérilisée, desséchée puis imbibée (jusqu'à H° ≏ 7 %) d'un extrait aqueux de terre de mil.

E4 — Terre infestée entre 2 lots de terre de mil, terre lavée (H° ramenée à 7 %).

Les taux de déplacement dans ces quatre colonnes furent les suivants:

E1: 0,1 %
E2: 13,3 %
E3: 1,6 %
E4: 22,7 %

En conclusion:

— l'extrait aqueux de terre de mil n'a aucune action favorisante sur le déplacement.

— la terre de mil, lavée, favorise même mieux les déplacements que la terre de mil naturelle. Nous reviendrons sur ce point.

— si donc existe dans le sol cultivé en mil une substance favorisant les déplacement des nématodes, celle-ci doit être adsorbée sur certaines particules puisqu'elle n'est pas éluable.

Adsorption dans le sol

On sépare, grâce à l'élutriateur, la fraction argileuse de la fraction sableuse, d'une part de terre stérilisée, d'autre part de terre de mil; les différentes fractions sont séchées ($H° \backsimeq 1\ \%$); puis on recompose des sols artificiels et, leur humidité amenée à 7 %, on teste le déplacement dans les 3 cas suivants:

	Taux de déplacement
— argile terre de mil + sable terre stérilisée	19,7 %
— argile terre stérilisée + sable terre de mil	0,3 %
— terre de mil naturelle (séchée à $H° \backsimeq 1\ \%$ puis ramenée à 7 %)	16,2 %

D'après ces chiffres c'est donc la fraction argileuse du sol qui est active.

Action de la chaleur

Du sol ayant porté du mil est chauffé à 90-95°C pendant 10 mn.; on teste ensuite le déplacements de la façon suivante:

La terre à tester est disposée en deux parties, au dessus et en dessous, d'une terre infestée par *H. paradoxa,* dans des colonnes verticales, plus petites que celles utilisées précédemment (diam.: 5 cm; hauteur: 35 cm). Dans ces colonnes le déplacement de *H. paradoxa* a une valeur plus faible que dans les grandes colonnes. Dans la terre de mil la moyenne est de 3,3 %, dans la terre stérile de 0,2 %. On peut admettre qu'un déplacement inférieur à 0,9 % ne révèle aucune action favorisante.

Résultats:

	Taux de déplacement
— terre de mil chauffée	0,8 %
— terre de mil naturelle	6,3 %
— terre stérilisée témoin	0,5 %

Le facteur favorisant les déplacements est donc thermolabile.

Résistance à la dessication

De la terre de mil est desséchée à l'exsiccateur pendant 16 jours ($H° \backsimeq 0{,}2\ \%$); elle est ramenée ensuite à $H° \pm 7\ \%$ puis testée. L'attirance reste positive (déplacement: 1,8 %); de même 15 jours après (3,9 %), mais non un mois plus tard (0,5 %).

Rémanence dans le sol

Deux lots de terre de mil sont conservés, l'un à sec (H° ≏ 1 %), l'autre à H° ≏ 7 %.

Les déplacement sont testés tous les mois:

— au deuxième mois pour les deux lots, l'attirance est positive (respectivement 5,4 % et 1,3 %).

— au troisième mois seule la terre conservée à sec est positive (1,6 % et 0,3 %).

— au quatrième mois les deux sont négatives (0,8 % et 0,05 %).

Action à distance du système radiculaire du mil

— Horizontalement:

Dans un bac de 1 m de diamètre, on place au centre un panier de 20 cm de diamètre et de 40 cm de hauteur réalisé avec de la toile de tamis de 250 meshes/inch. Le bac et le panier sont remplis de terre stérilisée. On cultive pendant 6 mois du mil à intérieur du panier; les racines ne peuvent passer à l'extérieur. On prélève le sol du bac de 10 cm en 10 cm en s'éloignant du panier; puis on teste le déplacement dans ces échantillons de sol:

	Taux de déplacement
10 cm du panier	4,2 %
20 cm du panier	5,6 %
30 cm du panier	4,2 %
40 cm du panier	3,7 %

— Verticalement:

On cultive du mil dans un tube vertical de plexiglas de 10 cm de diamètre et de 100 cm de haut séparé en deux, à 30 cm de l'extrémité supérieure, par une toile de tamis de 250 meshes/inch. Les racines du mil ne peuvent donc descendre plus loin que 30 cm. Après trois mois on fragmente la portion inférieure de la colonne de terre en 7 portions correspondant chacune à 10 cm de haut, et on teste le déplacement dans chacune de ces fractions:

	Taux de déplacement
0 à 10 cm des racines	5,8 %
10 à 20 cm des racines	6,3 %
20 à 30 cm des racines	5,3 %
30 à 40 cm des racines	4,5 %
40 à 50 cm des racines	3,8 %
50 à 60 cm des racines	?
60 à 70 cm des racines	2,9 %

On voit donc d'après ces deux types d'expériences que l'action du système radiculaire de la plante sur le sol, au point de vue de son influence sur le déplacement des nématodes, n'est pas limitée à la rhizosphère proche mais se fait sentir à distance, tant horizontalement que verticalement.

Essais avec plantes non hôtes

Diverses légumineuses, plantes non hôtes pour *H. paradoxa*, ont été cultivées sur sol stérilisé; puis, après 3 mois, le déplacement a été testé. Résultats:

	Taux de déplacement
Cassia occidentalis L.	0,5 %
Cassia corymbosa Lam.	0,2 %
Cassia mimosoides L.	0,3 %
Centrosema plumieri Benth.	3,2 %
Centrosema pubescens Benth.	0,4 %
Clitoria ternatea L.	0,4 %
Crotalaria astragalina Hochst.	1,2 %
Crotalaria juncea L.	0,2 %
Crotalaria usaramoensis Bak.	0,6 %
Dolichos carabamcito?	1,4 %
Indigofera endecaphylla Jacq.	0,6 %
Moghania macrophylla Willd.	0,5 %
Mucuna pruriens D.C. var. *utilis* (Wall) Bak. ex Burck.	2,0 %
Tephrosia vogelii Hook.	0,4 %

D'après ces chiffres les déplacements dans ces sols de rhizosphère de Légumineuses sont, en général, beaucoup moins importants que dans ceux de rhizosphère de mil.

Facteur(s) défavorisant le déplacement

Nous avons déjà vu que le lavage d'une terre de mil y rend le déplacement de *H. paradoxa* plus important. On peut supposer qu'il existe dans le sol cultivé en mil un ou des facteurs défavorisants, hydrosolubles.

L'expérience suivante a donc été montée: de la terre de mil est divisée en 3 lots: le premier est laissé quel (H° ⏜ 7 %); le second est lavé par plusieurs passages de la même eau distillée, séché, puis ramené à H° ⏜ 7 % avec de l'eau distillée; le troisième lot est desséché puis ramené à H° ⏜ 7 % avec l'eau ayant servi à laver le deuxième. On teste ensuite le déplacement de *H. paradoxa* dans chaque lot. Les résultats pour 3 lots de terre différents ont été les suivants:

	Terre de mil naturelle	Terre de mil lavée	Terre de mil + eau de lavage
1	1,6 %	5,1 %	0,2 %
2	2,4 %	3,1 %	1,4 %
3	3,7 %	3,8 %	3,3 %

Malgré la variabilité des résultats, on voit que, au point de vue du déplacement de *H. paradoxa*, les 3 catégories de traitements se classent toujours dans le même ordre. Cela semble une indication sérieuse de la présence dans le sol ayant porté du mil d'un ou plusieurs facteurs, hydrosolubles, défavorisant les déplacements de *H. paradoxa.*

Un principe inhibiteur analogue a déjà été mis en évidence par Seinhorst (1950) dans des sols cultivés de Hollande. Ce principe, thermolabile, inhibait les mouvements de *Ditylenchus dipsaci* (Kühn).

Remarques:

La grande variabilité des résultats obtenus dans le même type d'expérience provient de plusieurs facteurs:

— les lots de terre de mil sont de constitution physique identique, mais la végétation du mil n'est jamais équivalente d'un pot à un autre. De plus, ces pots étant sous abri, mais à l'air libre, les influences climatiques peuvent se faire sentir.

— la terre infestée par *H. paradoxa* qui sert à tester le déplacement est elle-même une terre de mil. Il y a donc interaction avec la terre à tester.

— le tassage de la terre dans les colonnes, aussi soigneusement soit-il réalisé, peut ne pas être identique d'une colonne à l'autre.

L'essentiel est que dans la même série comparative d'expériences, les résultats, variables au point de vue du chiffre même des déplacements, se classent dans le même ordre pour les différents traitements.

Cette variabilité est inévitable si l'on tient à se placer, comme nous l'avons fait, dans des conditions aussi voisines que possible des conditions naturelles.

CONCLUSIONS

En conclusion, les expériences précédentes ont apporté des indications sur les faits suivants:

— les déplacements verticaux de *Hemicyliophora paradoxa* Luc sont favorisés par un courant ascendant d'humidification; il ne s'agit pas seulement d'un entraînement passif.

— les déplacements verticaux sont plus importants de haut en bas que de bas en haut.

— la culture d'une plante hôte, en l'occurence du mil (*Pennisetum typhoides*) sur du sol préablement stérilisé favorise le déplacement de *Hemicycliophora paradoxa* par rapport au sol stérilisé primitif.

— Le ou les facteurs favorisant ces déplacements et provenant de la culture du mil:
  - — ne sont pas éluables du sol
  - — sont adsorbés sur la fraction argileuse
  - — sont thermolabiles
  - — résistent à la dessication
  - — sont doués d'une certaine rémanence, plus grande pour un sol desséché ($H° \backsimeq 1\ \%$) que pour un sol humide ($H° \backsimeq 7\ \%$)
  - — se retrouvent à des distances appréciables du système radiculaire du mil, tant verticalement qu'horizontalement.

— La culture de certaines Légumineuses, plantes non hôtes, ne semble pas favoriser les déplacements de *H. paradoxa.*

— Un ou des facteurs défavorisant les déplacements existent dans les sols cultivés en mil. Ce ou ces facteurs sont hydrosolubles.

SUMMARY

Preliminary note on the migration of Hemicycliophora paradoxa *Luc in the soil*

The vertical migration of *Hemicycliophora paradoxa* Luc from an infested soil to non-infested soil was studied. The infested soil was taken from the rhizosphere of the pearl millet *Pennisetum typhoides*. Non-infested soil was either sterilized, plain soil, or sterilized soil on which pearl millet had grown, free of nematodes, for 2 months and is referred to as "millet soil".

Columns of infested and non-infested soil were placed one above the other in vertical perspex tubes. In one type of experiment the moisture content was kept relatively constant (H = ± 7 %), in the other type the moisture content changed from ± 5 % at the top to ± 20 % (subsaturated) at the bottom of the tube. The experiments ran for 5 days, after which the nematodes in each part of the tube were counted separately, and the numbers migrating recorded as percentage of total population remaining at the end of the experiment.

The results were as follows: —

Mean % migration of nematodes from infested to non-infested soils

	Infested soil placed at:		
	Top of tube	Bottom	Middle
Migration to sterilised soil	1.5 %	1.1 %	0.6 %
Migration to sterilised soil moisture gradient present	4.1 %	13.5 %	7.1 %
Migration to "Millet soil"	14.5 %	3.6 %	8.8 %
Migration to "Millet soil" moisture gradient present	19.8 %	36.1 %	36.2 %

It appears that *H. paradoxa* is stimulated to migrate when a moisture gradient exists and also that growing millet has a similar effect and that both sources of stimulation are additive.

Other experiments indicated that the factor(s) emanating from millet, could not be washed from the soil, were absorbed on the clay fraction, were thermolabile, resistant to drying and more residual in dry than wet soil. The factor(s) acted at a considerable vertical and horizontal distance from the millet roots.

Fourteen legumes (non hosts for *H. paradoxa*) tested showed no evidence of any stimulatory action in sterilized soil.

Millet roots also appear to produce one or several water soluble factors, inhibitory to migration of *H. paradoxa*.

BIBLIOGRAPHIE

PETERS, B. G. (1953). Vertical migration of potato root eelworm — *J. Helm.* **27**, 107-112.

SEINHORST, J. W. (1950). De betekenis van de toestand van de grond voor het optreden van aantasting door het stengelaaltje (*Ditylenchus dipsaci* (Kühn), Filipjev). *Tijdschr. PlZiekt.* **56**, 289-349.

WALLACE, H. R. (1959). Movement of eelworms. IV. The influence of water percolation. *Ann. appl. Biol.* **47**, 131-139.

—— (1960). Movements of eelworms. VI. The influence of soil type, moisture gradients and host plant roots on the migration of the potato root eelworm, *Heterodera rostochiensis* Wollenweber. *Ann. appl. Biol.* **48**, 107-120.

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XIPHINEMA DE L'OUEST AFRICAIN (NEMATODA-DORYLAIMOIDEA) DEUXIÈME NOTE [1])

PAR

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L'auteur donne la description et le dessin de six nouvelles espèces de *Xiphinema* provenant de l'Ouest Africain: *X. attorodorum* n. sp., *X. flagellicaudatum* n. sp., *X. longicaudatum* n. sp., *X. ifacolum* n. sp., *X. nigeriense* n. sp., et *X. longidoroides* n. sp. *X. ensiculiferum* (Cobb, 1893) Thorne, 1937 est redécrit et un néotype désigné.

Cet article donne la description de six nouvelles espèces de *Xiphinema* dont cinq ont été rencontrées ces deux dernières années au cours de nombreux examens de sols de Côte d'Ivoire, du Togo et de Guinée. La dernière, provenant de Nigeria, nous a été transmise par F. E. Caveness.

La plus fréquente parmi ces six espèces avait été rapportée dans deux articles antérieurs (Luc 1957 et 1958) à *Xiphinema campinense* Lordello, 1951. Le matériel type de cette dernière espèce n'est malheureusement pas consultable, mais des échantillons de *X. campinense* identifiés comme tels par L. E. G. Lordello et envoyés de Floride par A. C. Tarjan nous ont montré suffisamment de différences avec les specimens de l'Ouest Africain pour que l'on doive considérer ceux-ci comme appartenant à une espèce distincte et nouvelle, décrite ici sous le nom de *Xiphinema attorodorum* n. sp.

Cet article comporte également une redescription de *Xiphinema ensiculiferum* (Cobb, 1893) Thorne, 1937.

C'est volontairement que peu de détails ont été donnés sur la structure de la gonade femelle. Ce point particulier fait l'objet d'une autre publication (Luc, 1961) portant sur plus de vingt espèces (dons les six décrites ou redécrites ici) provenant de l'Ouest Africain, des U.S.A. et de l'Inde.

XIPHINEMA ATTORODORUM n. sp.

Xiphinema campinense in Luc, 1957; Luc, 1958; = *Xiphinema* n. sp. a in Luc & de Guiran, 1960; Luc & Hoestra, 1960; non: *Xiphinema campinense* Lordello, 1951.

Dimensions: 10 ♀♀: L = 2,49-2,81 mm (moy. = 2,61 mm); a = 51,8-73,3; b = 6,1-8,5; c = 29,4-44,4; V = $^{7,9\text{-}12,3}$ 40,1-42,0 % $^{7,6\text{-}12,5}$

[1]) Présenté à la "1ère Conférence Interafricaine de Nématologie", Nairobi, 24-28 Octobre 1960.

Holotype: Femelle: L = 2,622 mm; a = 63,9; b = 6,9; c = 44,4; V = ^{8,9} 40,4% ^{8,4}

Mâle (allotype): L = 2,760 mm; a = 50,1; b = 7,5; c = 39,4; T = ?

Description:

Femelle: Habitus à peine courbé. Corps cylindrique peu effilé vers l'avant, un peu plus vers l'arrière. Cuticule très finement striée transversalement; ces striations non visibles sur tous les individus. Pores latéro-subdorsaux, latéro-subventraux et cervicaux présents, pores ventraux présents dans la partie postérieure du corps. Champ latéral occupant environ ¼ du diamètre au milieu du corps.

Lèvres arrondies, soudées, séparées du corps par une incisure moyenne. Ouverture des amphides dans cette incisure et occupant 75 à 90% de la largeur correspondante.

Stylet typique du genre, long de 184-193 μ, la partie épaissie étant longue de 68-76 μ (36,9-40,0% de la longueur totale du stylet). Guide tubulaire long de 11-28 μ.

Portion antérieure de l'oesophage étroite, repliée sur elle-même et comportant dans la paroi musculeuse une petite pièce chitinisée triangulaire, à 20-35 μ en arrière de la base du stylet. Bulbe oesophagien musculeux long de 83-102 μ (19,4-31,8% de la longueur oesophagienne). Cardia large et plat.

Vulve en croix, à 41% en moyenne. Gonades femelles réfléchies, longues de: l'antérieure 208-308 μ, la postérieure 208-314 μ.

Queue régulièrement conique, à extrémité arrondie, longue de 59-89 μ (2,8-3,3 largeurs anales). La queue porte 3 paires de pores, la postérieure, médiane, à 18-30 μ de l'extrémité caudale; la seconde, subdorsale, à 36-54 μ de l'extrémité caudale; la position de la paire antérieure peut varier de 14 μ en avant de l'anus à 15 μ en arrière. Il existe une quatrième paire en avant de l'anus; bien que sur certains individus dont la troisième paire est située assez loin en arrière de l'anus, cette quatrième paire soit située presque au niveau de celui-ci, on ne peut proprement la qualifier de caudale. La cuticule de la queue est épaissie subterminalement, ne laissant libre qu'un fin canalicule assez caractéristique.

Mâle: Habitus presque rectiligne, partie postérieure seule recourbée en crochet. Corps cylindrique, longuement effilé vers l'avant, peu vers l'arrière.

Région labiale identique à celle de la femelle. Stylet long de 183 μ; partie basale longue de 64 μ (34,9% de la longueur du stylet). Pièce chitinisée noyée dans la musculature oesophagienne à 27 μ en arrière de la base du stylet. Bulbe oesophagien long de 87 μ (23,8% de la longueur oesophagienne) et large de 17 μ.

Spicules présentant une partie distale légèrement renflée en tête, puis une portion rectiligne suivie d'une partie coudée amincie à son extrémité; longueur des spicules (à la corde): 48 μ. Pas de gubernaculum visible; un repli cuticulaire intérieur, assez épais, de la lèvre postérieure de l'anus semble pouvoir en jouer le rôle. Il n'existe aucun supplément adanal, ventral ou ventro-latéral. Nous n'avons

Fig. 1. *Xiphinema attorodorum* n. sp., femelle; a: portion antérieure; c, d, e: différents types de queue; b: mâle: spicules et queue. *Xiphinema flagellicaudatum* n. sp., femelle; f: portion antérieure; g: queue. (Gross. × 570).

pu observer les testicules; aucun spermatozoïde n'était non plus visible dans le canal déférent.

Queue régulièrement conique, longue de 70 μ (2,2 largeurs anales); cuticule épaissie subdistalement comme chez la femelle; une seule paire de pores caudaux, latéro-subdorsaux, à 40 μ de l'extrémité caudale.

Lieu-type: Sol, voisinage des racines de *Cocos nucifera* L. Akodessewa — Togo.

Holotype: Femelle — Lame 27-To-170 — Laboratoire de Nématologie — — I.D.E.R.T. — Abidjan — Côte d'Ivoire.

Allotype: Mâle — Lame 44-To-148. — Laboratoire de Nématologie — I.D.E.R.T. — Abidjan — Côte d'Ivoire.

Diagnose: Parmi les espèces didelphiques à queue courte (moins de 4 largeurs anales), les femelles de *Xiphinema attorodorum* n. sp. sont caractérisées par la queue portant 3 paires de pores caudaux et de forme régulièrement conique; ce dernier caractère suffit à la séparer de *X. pratense* Loos, 1949 et de *X. setariae* Luc, 1958 qui toutes deux possèdent une queue conique-subdigitée beaucoup plus courte. *X. attorodorum* n. sp. est assez différent de *X. campinense* Lordello, 1951 qui possède une queue irrégulièrement conique (subdigitée) portant 4 paires de pores caudaux, la quatrième étant parfois antérieure à l'anus; il existe en plus chez cette espèce 2 autres paires de pores toujours situées antérieurement à l'anus; enfin l'épaississement subterminal de la cuticule caudale de *X. attorodorum* n. sp. ne se retrouve pas chez *X. campinense* Lordello, 1951. Le mâle est unique dans le genre par l'absence de tout supplément.

L'étude de populations de *Xiphinema attorodorum* n. sp. provenant de régions variées de l'Ouest Africain et de la rhizosphère de différentes plantes révèle une remarquable constance dans la forme et les dimensions des différents organes. Seule varie très légèrement la longueur totale des femelles, les moyennes extrêmes (sur 10 individus) étant de 2,610 mm pour la population type provenant de la rhizosphère de cocotier au Togo et de 2,914 mm pour des individus prélevés au voisinage de racines de *Coffea canephora* Pierre en Côte d'Ivoire.

Une seule population, observée au voisinage de racines de *Citrus vulgaris* Risso à Foulaya (Guinée) en mélange avec *X. ifacolum* n. sp., diffère du type par un caractère plus important: la queue est en effet un peu plus longue, mesurant 82-98 μ (3,2-3,7 largeurs anales) au lieu de 59-89 μ (2,8-3,3 largeurs anales); de plus c'est dans cette dernière population, et dans elle seule, qu'ont été reconnus des individus à hermaphrodisme protandre qui seront décrits dans un article ultérieur (Luc, 1961).

Notons que dans les populations rencontrées dans la rhizosphère du cocotier au Togo, parmi près de 2000 femelles examinées nous n'avons observé qu'un seul mâle. Encore celui-ci ne semblait-il pas fonctionnel, car il nous a été impossible de découvrir la moindre trace de gonade.

XIPHINEMA FLAGELLICAUDATUM n. sp.

= *Xiphinema* n. sp. b in Luc & de Guiran 1960; in Luc & Hoestra 1960.

Dimensions: 1 ♀ (holotype): L = 2,61 mm; a = 59,4; b = 7,2; c = 11,4; V = ^{12,7} 44,5% ^{10,7}.

Description:

Femelle: Habitus presque rectiligne (animal tué par la chaleur). Corps cylindrique, longuement effilé vers l'avant et surtout vers l'arrière. Cuticule finement striée transversalement. Champ latéral occupant environ $^1/_3$ du diamètre correspondant au milieu du corps. Pores latéro-subventraux et latéro-subdorsaux présents. Pores ventraux absents.

Lèvres soudées, arrondies, séparées du corps par une légère incisure. Ouverture des amphides au niveau de l'incisure, occupant les $^3/_4$ de la largeur correspondante.

Stylet typique du genre, long de 198 μ; partie basale épaissie longue de 77 μ (34 % de la longueur du stylet). Guide tubulaire long de 12 μ.

Oesophage à partie antérieure étroit, plusieurs fois enroulée sur elle-même; bulbe basal musculeux, long de 77 μ (21 % de la longueur oesophagienne) et large de 22 μ. Cardia conique, large et haut. Pièce chitinisée noyée dans la paroi oesophagienne en forme de pointe de flèche (5 × 1 μ), à 51 μ en arrière de la base du stylet.

Vulve située un peu en avant de la moitié du corps (44,5 %). Gonades femelles réfléchies, longues de: l'antérieure 334 μ, la postérieure 282 μ.

Queue régulièrement amincie, filiforme, très longue (230 μ ou 12 largeurs anales), se terminant en pointe; deux paires de pores caudaux, les pores gauches et droits non situés au même niveau.

Mâle: inconnu.

Lieu type: Sol, au voisinage des racines de *Cocos nucifera* L., entre Akodessewa et Bé — Togo.

Holotype: Femelle — Lame 45-To-154 — Laboratoire de Nématologie de l'I.D.E.R.T. — Abidjan — Côte d'Ivoire.

Diagnose: le grand développement de la queue dont la longueur équivaut à 12 largeurs anales suffit à distinguer *X. flagellicaudatum* n. sp. de toutes les espèces déjà décrites.

XIPHINEMA LONGICAUDATUM n. sp.

= *Xiphinema* n. sp. c in Luc & de Guiran, 1960

Dimensions: 1 ♀ (holotype): L. = 2,888 mm; a = 48,1; b = 5,6; c = 17,4; V = ^{2,6} 35,1 % ^{10,4}.

Description:

Femelle: Habitus en arc de cercle assez ouvert. Corps cylindrique, effilé vers l'avant, beaucoup plus longuement vers l'arrière. Cuticule finement striée transversalement. Champ latéral occupant environ $^1/_4$ du diamètre correspondant au milieu

du corps. Pores latéro-subventraux, latéro-subdorsaux et cervicaux présents; pores ventraux apparemment absents.

Lèvres soudées, arrondies, sans incisure les séparant du corps. Ouverture des amphides occupant 55 % de la largeur correspondante.

Stylet typique du genre, long de 232 μ dont 81 μ pour la partie basale élargie (35 % de la longueur du stylet). Guide tubulaire long de 13 μ.

Portion antérieure de l'oesophage étroite, enroulée sur elle-même avant le bulbe basal et comprenant, à 6 μ de la base du stylet, une pièce chitinisée triangulaire de 3 $\times$ 2 μ, noyée dans la paroi musculeuse. Bulbe basal musculeux long de 90 μ (18 % de la longueur oesophagienne) et large de 29 μ. Cardia conique, large et plat.

Vulve située environ au tiers antérieur (35,1 %). Gonade postérieure normalement développée, réfléchie, longue de 300 μ. Gonade antérieure atrophiée, droite, sans ovaire, longue de 75 μ.

Queue d'abord conique puis effilée, à extrémité arrondie, longue de 166 μ (6,3 largeurs anales); 2 paires de pores caudaux, les pores gauches et droits n'étant pas situés au même niveau: pores gauches à 3 μ et 24 μ en arrière de l'anus, pores droits à 2 μ et 14 μ.

Mâle: inconnu.

Lieu type: Sol, au voisinage de racines de *Piper nigrum* L.; Plantation Bafécao — M'Brimbo — Côte d'Ivoire — (Rec. J. W. Seinhorst).

Holotype: Femelle — Lame 140-CI-216 — Laboratoire de Nématologie — I.D.E.R.T. — Abidjan — Côte d'Ivoire.

Diagnose: Parmi les espèces à tractus génital femelle double et queue mesurant entre 5 et 7 largeurs anales, *X. longicaudatum* n. sp. se différencie de *X. hallei* Luc, 1958 par sa vulve plus antérieure (35,1 % contre 46,7 %), sa queue semblable de forme mais ne comportant que 2 paires de pores caudaux au lieu de trois et par l'absence d'incisure céphalique. Ce dernier caractère la sépare également de *X. insigne* Loos, 1949, de même que le nombre de pores caudaux (2 paires contre 7) et la plus grande longueur du stylet (232 μ contre 158 μ). *X. longicaudatum* n. sp. se différencie enfin de *X. indicum* Siddiqi, 1959, espèce ayant également une gonade femelle antérieure réduite, par sa queue beaucoup plus longue (166 μ contre 82 μ) et ne comportant que deux paires de pores caudaux au lieu de trois ainsi que par son stylet plus long également (232 μ contre 158-167 μ).

XIPHINEMA ENSICULIFERUM (Cobb, 1893) Thorne, 1937

Xiphinema ensiculiferum (Cobb, 1893) Thorne, 1937 a été récolté par Cobb, en 1891, au voisinage de racines de *Musa* sp., aux Iles Fidji. Cette espèce n'a été rencontrée depuis, à notre connaissance, qu'en trois occasions: par Loos (1949) à Ceylan dans la rhizosphère de *Lilium longiflorum* Thunb. et de graminées indéterminées, par Carvalho (1955) au Brésil dans la rhizosphère d'une plante ornementale indéterminée et par Williams (1959) à l'île Maurice dans la rhizosphère de canne à sucre. Loos donne une description des femelles et des mâles, les-

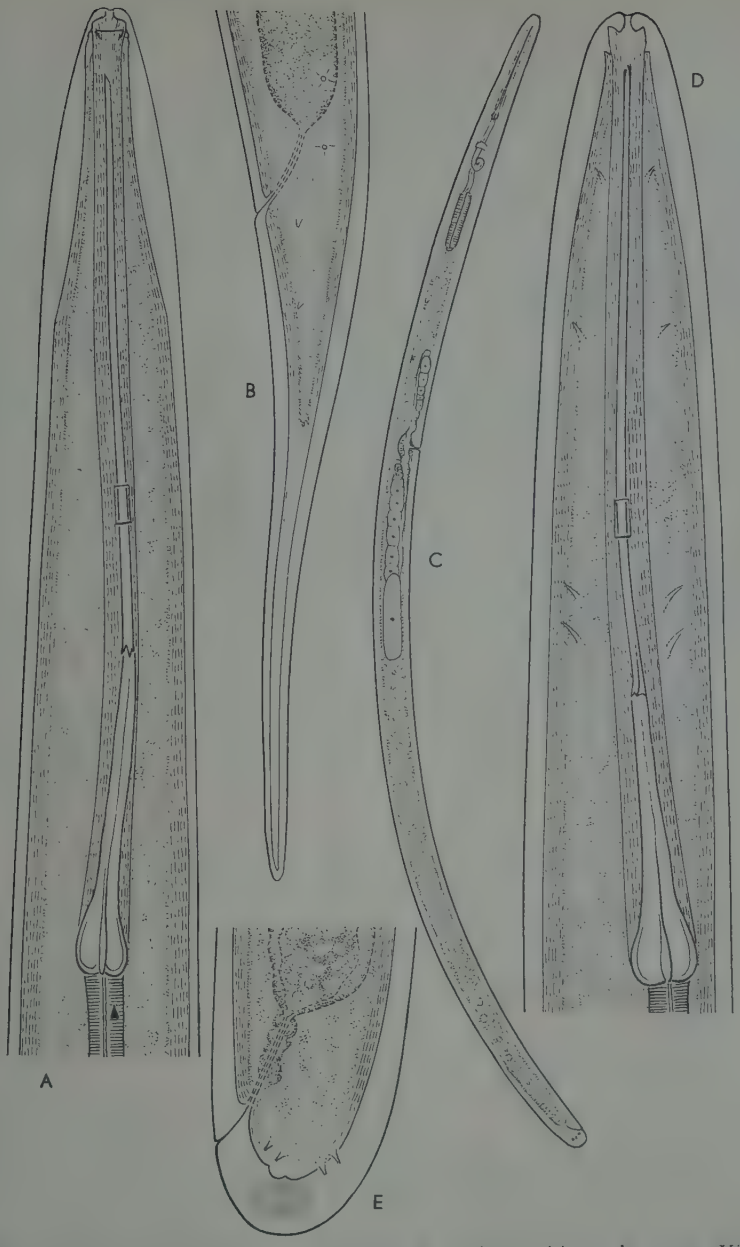

Fig. 2. *Xiphinema longicaudatum* n. sp., femelle; a: portion antérieure; b: queue. *Xiphinema ensiculiferum* (Cobb, 1893) Thorne, 1938, femelle; c: vue en entier; d: portion antérieure; e: queue (Gross. a, b, d, e: × 570; c: × 170).

quels n'avaient pas été observés par Cobb. Carvalho donne également une description des femelles rencontrées au Brésil.

Un certain nombre de différences séparent les femelles observées par Loos de la description originale: lèvres en bourrelet applati et incisure céphalique bien marquée, stylet nettement plus petit, queue non parfaitement hémisphérique; les femelles rencontrées par Carvalho sont, par contre, tout à fait conformes aux données de Cobb, mais leur description est assez sommaire. En Côte d'Ivoire, *X. ensiculiferum* a été rencontré, en petit nombre, dans la rhizosphère de *Musa acuminata* Colla. var. Poyo (= *M. sinensis* Sajot var. Poyo) et de *Ravenala madagascariensis* Gmel., autre Musacée.

Les femelles examinées sont identiques à la description originale, notamment par l'aspect de la région labiale et les dimensions du stylet; seule la question de la gonade antérieure semble plus délicate: Cobb ne donne aucun détail sur la structure de l'appareil génital mais sa formule chiffrée suggère qu'il n'existe qu'une seule gonade, postérieure; ceci est d'ailleurs repris par Thorne (1939). Loos décrit chez les individus de Ceylan une „gonade antérieure courte et rudimentaire variant en longueur de ¼ à une largeur du corps"; Carvalho décrit également une gonade antérieure régressée, droite. Les individus rencontrés de Côte d'Ivoire possèdent par contre une gonade antérieure réfléchie, beaucoup plus courte et plus grêle que la gonade postérieure mais de structure normale, avec une partie ovarienne développée. Cobb parle à propos des figures qui illustrent la description originale d'une femelle immature; de ce fait la structure et même l'existence de la gonade antérieure ont pu fort bien passer inaperçues.

Nous pensons qu'il est bon de donner, dans ces conditions, une description complète des individus de *X. ensiculiferum* rencontrés en Côte d'Ivoire, et de désigner un néotype, ni Loos ni Carvalho n'y ayant procédé, et l'hôte présumé étant le même pour les individus de Cobb et les nôtres.

XIPHINEMA ENSICULIFERUM (Cobb, 1893) Thorne, 1937

Dimensions: 7 ♀♀: L = 1,81-2,38 mm, a = 30,2-35; b = 3,6-6,2; c = 81,6-112,8, V = $^{9,1-10}$ 36,3-39,2 % $^{14,3-27,7}$

Néotype: L = 2,38 mm; a = 35; b = 6,2; c = 95,2; V = ^{9,7} 36,3 % ^{27,7}.

Description:

Femelle: Habitus très légèrement courbé. Corps cylindrique, épais, trapu, très peu effilé vers l'avant, encore moins vers l'arrière. Cuticule finement striée transversalement, épaisse surtout vers l'avant et à l'extrémité de la queue; champ latéral occupant $^1/_5$ du diamètre du corps au milieu. Pores cervicaux, présents; pores latéro-subdorsaux, latéro subventraux et ventraux non observés.

Lèvres soudées, arrondies vers l'avant, sans incisure les séparant du reste du corps. Amphides non observées. Un cercle de 10 papilles (pores !) bien visible.

Stylet typique du genre, long de 227-260 μ; partie basale épaissie longue de

81-92 μ (32-36 % de la longueur du stylet). Ailettes bien marquées à renforcement marginal moyen. Guide tubulaire long de 7-18 μ.

Oesophage à partie antérieure étroite, enroulée sur elle-même; bulbe basal musculeux long de 96-102 μ sur 23-25 μ. Cardia bas et plat.

Vulve situé à 36,3-39,2 %; gonade postérieure réfléchie, longue de 292-660 μ; gonade antérieure réfléchie plus courte, beaucoup plus grêle, longue de 172-232 μ. Oeufs développés vus uniquement dans la gonade postérieure; dimensions: 217 × 46 μ.

Queue parfaitement hémisphérique (0,5-0,6 largeur anale). Cuticule très épaissie à l'extrémité de la queue; quatre paires de pores caudaux, très souvent groupées deux à deux un peu postérieurement au niveau de l'anus; chez deux femelles un des pores était situé peu antérieurement à l'anus.

Juvénile: Ils ne présentent aucun caractère particulier, sinon que leur queue n'est pas parfaitement hémisphérique, mais plutôt en ogive basse.

Lieu type: Sol — voisinage des racines de *Musa acuminata* Colla. var. Poyo — Sassandra — Côte d'Ivoire (Réc. G. de Guiran).

Néotype: Lame 556-CI-308 — Laboratoire de Nématologie — I.D.E.R.T. — Abidjan — Côte d'Ivoire.

Diagnose: parmi les rares espèces à queue hémisphérique, *X. ensiculiferum* se distingue de *X. yapoense* Luc, 1958 par un ovaire antérieur beaucoup moins développé que l'ovaire postérieur, l'absence d'incisure céphalique, une taille plus faible et la présence de quatre paires de pores caudaux au lieu de deux. *X. ensiculiferum* présente de nombreux points communs avec *X. obtusum* Thorne, 1951 dont il diffère essentiellement par l'inégal développement des ovaires et la position plus antérieure de la vulve.

XIPHINEMA IFACOLUM n. sp.

= *Xiphinema* n. sp. d in Luc & de Guiran, 1960.

Dimensions: 10 ♀♀: L = 3,120-3,712 mm (moy. = 3,492 mm); a = 50,3-62,0; b = 7,3-11,3; c = 45,4-59,1; V = $^{12,2-31,6}$ 48,3-53,3 % $^{12,5-23,3}$

1 ♂ (allotype): L = 3,20 mm a = 62,9; b = 6,7; c = 43,9; T = ?

Holotype: Femelle: L = 3,538 mm; a = 59,9; b = 9,1; c = 55,1; V = ^{13,2} 51,0 % ^{12,5}.

Description:

Femelle: Habitus en arc de cercle largement ouvert. Corps allongé, cylindrique, régulièrement aminci vers l'avant et vers l'arrière. Cuticule très finement striée transversalement. Champ latéral occupant environ 1/4 du diamètre correspondant au milieu du corps. Pores latéro-subventraux, latéro-subdorsaux et cervicaux présents; pores ventraux présents dans la partie postérieure du corps.

Lèvres arrondies, soudées, séparées du corps par une très faible dépression

Ouverture des amphides dans cette dépression occupant les $^2/_3$ de la largeur correspondante.

Stylet long de 185-197 μ, la partie basale étant longue de 52-79 μ soit 33,4-40,6 % de la longueur du stylet; les trois ailettes présentent des renflements marginaux assez peu marqués. Guide tubulaire long de 12-15 μ.

Partie antérieure de l'oesophage étroite, repliée en boucle, comportant dans la partie musculeuse une pièce chitinisée triangulaire, pleine, de 2-3,5 μ de long sur 1,5-2,5 μ de large et située de 12 à 72 μ en arrière de la base du stylet. Bulbe basal musculeux long de 68-120 μ (21,7-30,4 % de la longueur oesophagienne) et de 17-26 μ de large. Cardia conoïde.

Vulve en fente profonde, au milieu du corps environ (48,3-53,3 %). Gonades réfléchies, comportant un organe Z très différencié; longueur de la gonade antérieure: 464-1121 μ; longueur de la gonade postérieure: 441-829 μ. Oeufs pouvant atteindre jusqu'à 325 μ de long.

Queue irrégulièrement conique, amincie à l'extrémité, presque subdigitée, de 59-75 μ de long (1,6-1,9 largeurs anales). Il existe trois paires de pores que l'on peut proprement qualifier de caudaux: la postérieure est située en position médiane, à moitié de la queue ou un peu postérieurement; les deux autres paires sont situées, en position médiane à dorso-latérale, de façon très variable entre la paire postérieure et l'anus, l'une d'entre elles pouvant même être située légèrement antérieurement à l'anus. Il existe une quatrième paire de pores, toujours située antérieurement à l'anus, que l'on ne peut donc qualifier de caudale.

Mâle: De même que pour *X. attorodorum* n. sp., parmi des centaines de femelles un seul mâle a été rencontré; il ne semble pas fonctionnel car, si les spicules sont bien formés, il est impossible d'observer le tractus génital et notamment les testicules.

Habitus rectiligne dans la portion antérieure, spiralé pour la portion postérieure du corps. Cuticule et pores comme chez la femelle.

Lèvres identiques à celles de la femelle. Stylet long de 194 μ dont 71 μ pour la portion basale. Guide tubulaire long de 14 μ.

Oesophage et intestin identiques à ceux de la femelle.

Spicules légèrement arqués, massifs, épais, longs de 53 μ à la corde. Pas de gubernaculum. Suppléments sexuels: une paire de suppléments ventraux un peu en avant de l'anus et 3 autres ventraux également situés à quatre longueurs de queue en avant de l'anus; 5 paires de suppléments subventraux, régulièrement espacés en avant de l'anus. Canal déférent à peine visible, sans spermatozoïdes; testicules apparemment absents.

Queue conique subdigitée, à cuticule épaissie surtout dorsalement, longue de 73 μ (1,8 largeurs anales). 3 paires de pores caudaux, la postérieure médiane, les deux autres médiodorsales; une quatrième paire, un peu en avant de l'anus, en position médiane.

Lieu type: Femelle — Sol, voisinage des racines de *Citrus vulgaris* Risso — Station I.F.A.C. — Foulaya près Kindia — Guinée.

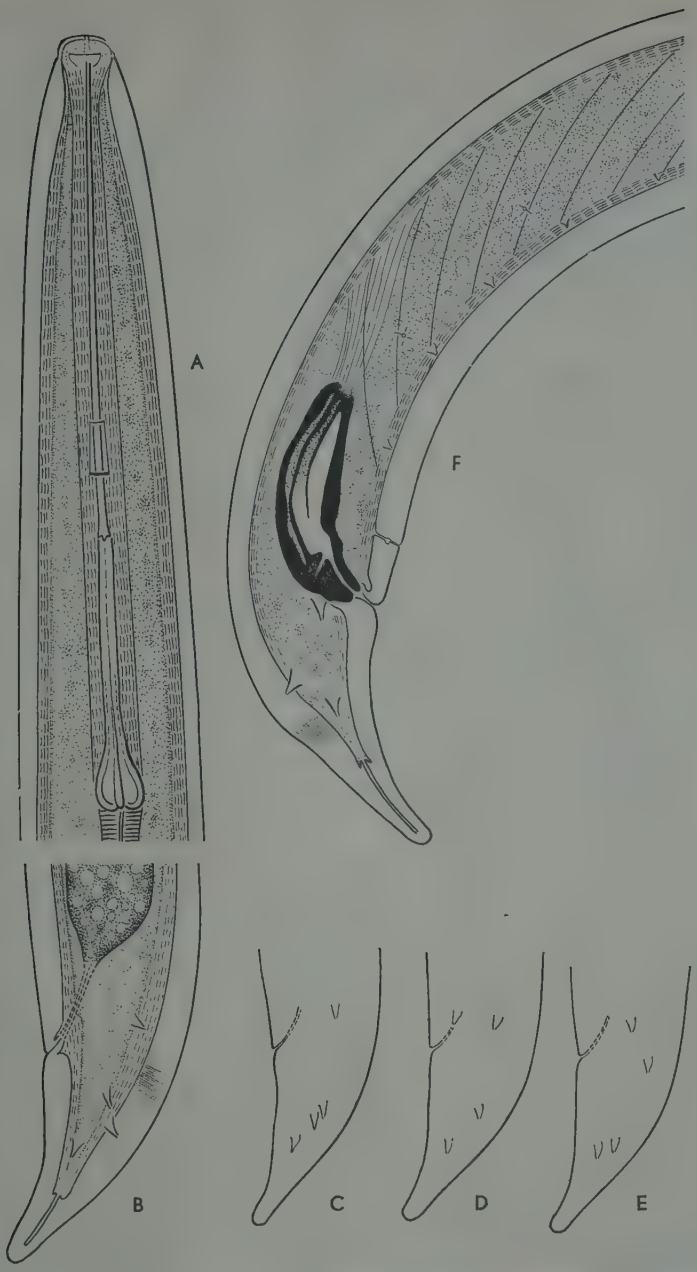

Fig. 3. *Xiphinema ifacolum* n. sp., femelle; a: portion antérieure; b: queue; c, d, e: variations dans la position des pores caudaux; mâle: f: partie postérieure. (Gross: a, b, f: × 570 — c, d, e: × 420).

Mâle — Sol, voisinage des racines de *Acalypha* sp. Pépinières de la ville d'Abidjan — Côte d'Ivoire.

Holotype: Femelle — Lame 305-Gu-221 — Laboratoire de Nématologie — I.D.E.R.T. — Abidjan — Côte d'Ivoire.

Allotype: Mâle — Lame 730-CI-397 — Laboratoire de Nématologie — I.D.E.R.T. — Abidjan — Côte d'Ivoire.

Diagnose: X. ifacolum n. sp. se différencie de l'autre espèce possédant un organe Z dans le tractus génital femelle, *X. ebriense* Luc, 1958, par la structure même de cet organe, la position postérieure de la vulve (48,3-53,3% contre 42,1-45,6%) et la forme de la queue, conique et non nettement digitée.

XIPHINEMA NIGERIENSE N. SP.

Dimensions: 10 ♀♀: L = 1,68-1,97 mm (moy. = 1,85 mm); a = 43,7-54,2; b = 4,3-7,8; c = 14,7-19,7; V = $^{16,1-27,7}$ 49,5-53 % $^{11,5-35,3}$

Holotype: Femelle: L = 1,83 mm; a = 48,3; b = 6; c = 19,7; V = ^{27,7} 51,6 % ^{35,3}.

Description:

Femelle: Habitus à peine courbé. Corps cylindrique longuement effilé vers l'avant et vers l'arrière. Cuticule très finement striée transversalement. Pores latéro-subdorsaux, latéro-subventraux et cervicaux présents; pores ventraux présents dans la partie postérieure du corps. Champ latéral occupant environ ¼ du diamètre au milieu du corps.

Lèvres soudées, arrondies vers l'avant; incisure peu marquée. Amphide large, à $^1/_2$ largeur labiale de l'avant et occupant des $^4/_5$ de la largeur correspondante.

Stylet typique du genre, mesurant 178-192 μ, la partie basale épaissie étant longue de 62-75 μ (33,8-38% de la longueur totale du stylet). Guide tubulaire long de 20 μ en moyenne.

Portion antérieure de l'oesophage étroite, parfois enroulée sur elle-même et comportant dans la paroi musculeuse une petite pièce chitinisée triangulaire, située à un emplacement très variable. Bulbe oesophagien long de 71-97 μ (20,5-26,1% de la longueur totale de l'oesophage. Cardia large et bas.

Vulve en croix située au milieu du corps environ (49,5-53,0%); gonades femelles réfléchies, longues de: l'antérieure: 272-534 μ; la postérieure: 200-649 μ. Oeufs longs de 168-176 μ et larges de 27 μ.

Queue longue (4,1-6,1 largeurs anales), arquée ventralement; partie antérieure conique suivie d'une portion cylindrique mince légèrement renflée en massue à l'extrémité; trois paires de pores caudaux; la postérieure en position médiane, située au ¼ de la longueur environ; les deux antérieures situées l'une au-dessus de l'autre, en position médiodorsale, la plus antérieure souvent au niveau de l'anus; les deux paires sont parfois contiguës, presque confondues; il n'existe pas d'autres pores avant l'anus.

Mâle: inconnu.

Lieu type: Sol, voisinage des racines de *Theobroma cacao* L.; ½ mile à l'Ouest de Omi Aboderin, 24 miles au Sud d'Ibadan — Western Nigeria (Réc. F. E. Caveness).

Holotype: Femelle — Lame 731-Ni-394 — Laboratoire de Nématologie — I.D.E.R.T. — Abidjan — Côte d'Ivoire.

Diagnose: X. nigeriense n. sp. est bien caractérisé par sa queue longue, arquée ventralement et légèrement massuée à l'extrémité. Il se différencie en outre de *X. longicaudatum* n. sp. par ses deux gonades également développées; de *X. insigne* Loos, 1949 par sa vulve postérieure (49,5-53% contre 29,8-31,6%); et de *X. hallei* Luc, 1958 par sa taille beaucoup plus faible (1,68-1,97 mm contre 3,52 mm).

XIPHINEMA LONGIDOROIDES N. SP.

= *Xiphinema* n. sp. e in Luc & de Guiran, 1960

Dimensions: 10 ♀♀: L = 2,865-3,920 mm (moy. = 3,373 mm); a = 57,7-77,4; b = 8-13; c = 46,2-61,8; V = $^{7,5-15,4}$ 52,1-55,6% $^{7,9-12,8}$.

Holotype: Femelle: L = 3,920 mm; a = 59,8; b = 9,3; c = 57,6; V = ^{11,6} 54,2% ^{7,9}.

Description:

Femelle: Corps cylindrique, longuement effilé vers l'avant et peu vers l'arrière. Cuticule très finement striée transversalement. Champ latéral occupant $^1/_3$ du diamètre correspondant au milieu du corps. Pores latéro-subventraux, latéro-subdorsaux et cervicaux présents, pores ventraux apparemment absents.

Lèvres soudées, arrondies, sans incisure marquée les séparant du corps, tout au plus note-t-on, chez certains individus, un rétrécissement léger et progressif à une distance d'une largeur labiale de l'avant. Amphide occupant 70% de la largeur correspondante.

Stylet long de 185-205 μ dont 58-69 μ (30-35,3 % de longueur du stylet) pour la partie basale élargie; les trois ailettes caractéristiques du genre que comporte le stylet à sa base sont nettes mais peu développées au contact avec la partie antérieure du stylet; elles s'affaiblissent en s'éloignant et les crêtes basales arrondies, épaissies sur leur bord que l'on observe chez les autres espèces sont remplacées par des arêtes fines à bord aminci. Guide tubulaire long de 15-19 μ.

Oesophage à portion antérieure étroite et repliée plusieurs fois sur elle-même de telle façon que le bulbe basal vienne presque au contact du stylet; pièce chitinisée noyée dans la paroi oesophagienne longue de 2,5-3 μ et large de 1-1,5 μ, située à 12-50 μ de la base du stylet. Bulbe basal musculeux long de 81-103 μ (22,3-37% de longueur oesophagienne) et large de 14-26 μ.

Vulve située un peu en arrière de la moitié du corps (52,1-55,6%). Gonades réfléchies; longueur de la gonade antérieure: 248-540 μ; gonade postérieure: 305-447 μ.

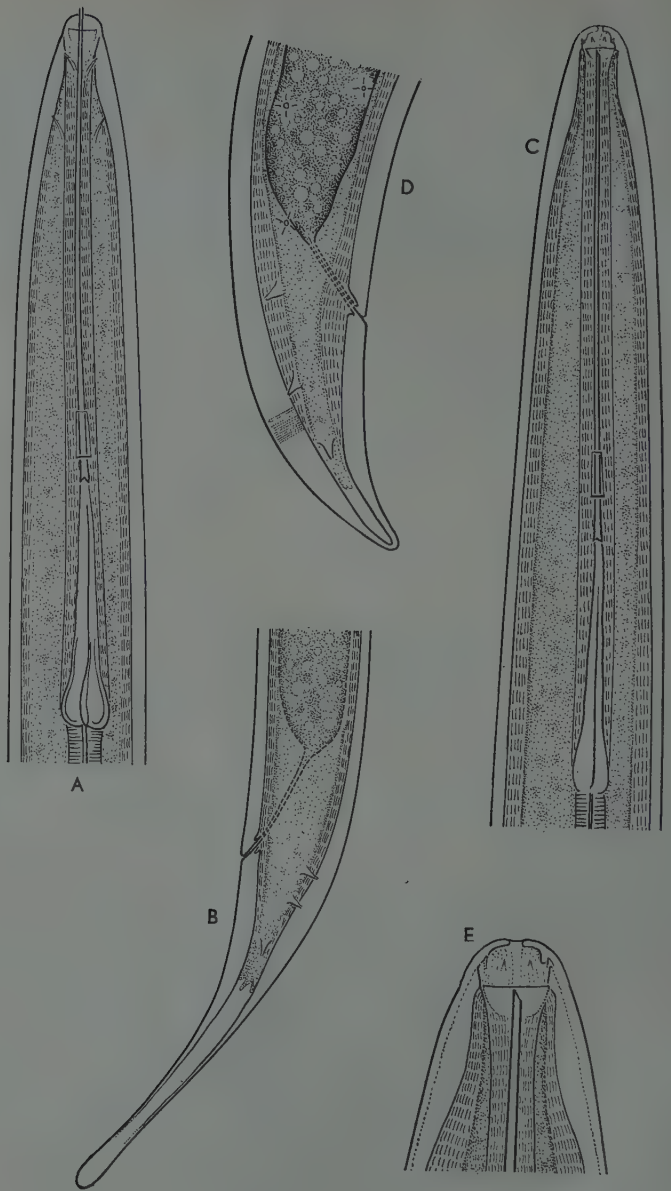

Fig. 4. *Xiphinema nigeriense* n. sp., femelle; a: partie antérieure; b: queue. *Xiphinema longidoroides* n. sp., femelle; c: partie antérieure; d: queue; e: région labiale. (Gross: a, b, c, d: × 570; e: × 1150).

Queue conique, légèrement courbée ventralement, extrémité arrondie, longue de 55-68 μ (1,8-2,2 largeurs anales). Trois paires de pores caudaux en position assez peu variable, la postérieure toujours latérale située à 31-37 μ de l'extrémité de la queue; la seconde, généralement subdorsale, à 44-61 μ de la queue; la troisième, antérieure, généralement subdorsale également, au niveau de l'anus, de 7 μ en avant à 2 μ en arrière de celui-ci. Les pores correspondant à une même paire ne sont que rarement situés exactement au même niveau; exceptionnellement, sur une seule femelle et sur un seul côté de celle-ci, nous avons observé un dédoublement du pore antérieur en un pore subdorsal et un pore subventral situés au même niveau.

Lieu type: Sol, au voisinage des racines de *Mapania superba* C.B. CL. — Forêt de Yapo — Côte d'Ivoire.

Holotype: Femelle: Lame 163-CI-202. Laboratoire de Nématologie. I.D.E.R.T. Abidjan. Côte d'Ivoire.

Diagnose: Xiphinema longidoroides n. sp. est nettement caractérisé par les ailettes de la base du stylet dépourvues d'épaississement marginal. Parmi les espèces voisines à queue conique courte et présentant 2 à 3 paires de pores caudaux, elle se distingue de *X. pratense* Loos, 1949, *X. setariae* Luc, 1958 et *X. attorodorum* n. sp. par sa vulve située à 48-55% et non au plus à 42%. Ce dernier caractère lui est commun avec *X. ifacolum* n. sp. et *X. americanum* Cobb, 1913; ces deux dernières espèces diffèrent de *X. longidoroides* n. sp., la première par la présence d'un organe Z, la seconde par la forme très différente de sa queue et son stylet beaucoup plus court (117 μ contre 194 μ, en moyenne).

Nous remercions le Dr. J. W. Seinhorst et M. G. de Guiran qui ont récolté une partie du matériel décrit, le Dr. A. C. Tarjan, le Dr. M. R. Siddiqi et le Dr. F. E. Caveness qui nous ont fait parvenir de nombreux échantillons de *Xiphinema* et enfin M. B. Souchaud qui a effectué les montages définitifs.

SUMMARY

Xiphinema *spp. from West Africa (Nematoda-Dorylaimoidea). Second Note.*

Six new species of *Xiphinema* from West Africa are described: — *X. attorodorum* n. sp. (earlier published as *X. campinense* Lordello) characterised by the vulva at 40-42 % and a relatively long regularly conical tail (59-98 μ, i.e. 2.8-3.7 anal-body-widths) with 3 pairs of caudal pores; only one male was found: *X. flagellicaudatum* n. sp. characterised by the longest tail in the genus (230 μ, i.e. 12 anal-body-widths): *X. longicaudatum* n. sp. which has a reduced anterior gonad without ovary and a long tail (166 μ, i.e. 6 anal-body-widths) with 2 pairs of pores: *X. ifacolum* n. sp. which has a Z organ in the female gonads, vulva at 48-53% and a short tail (1.6-1.9 anal-body-widths) with three pairs of pores; only one male was found: *X. nigeriense* n. sp. has a vulva at 49-53%, tail 4.1-6.1 anal-body-widths long, arcuate and slightly swollen at the top: *X. longidoroides* n. sp. characterised by the absence of thickening on the ridges of the stylet extension, so that their edges are difficult to see. *X. ensiculiferum* (Cobb, 1893) Thorne, 1937 is redescribed from a neotype.

BIBLIOGRAPHIE

CARVALHO, J. C. (1955). Plantas ornementais parasitadas por éspecies de gênero *Xiphinema* — *Rev. Inst. Lutz*, **15**, 180-185.

Cobb, N. A. (1893). Nematodes, mostly australian and fijian — *Macleay Mem. Vol., Linn. Soc. N.S.W.*, 252-308, pl. 36-42.

Loos, C. A. (1949). Notes on free living and plant-parasitic nematodes of Ceylon, No. 5 — *J. zool. Soc. India*, **1**, 23-29.

Lordello, L. E. G. (1951). *Xiphinema campinense*, nova especie (Nematoda, Dorylaimidae), *Bragantia*, **11**, 313-316.

Luc, M. (1957). Nématodes et „Maladie de Kaïncopé" du cocotier — *Oléagineux*, **12**, 791-793.

—— (1958). *Xiphinema* de l'Ouest Africain: description de cinq nouvelles espèces (Nematoda; Dorylaimidae) — *Nematologica*, **3**, 57-72.

—— (1961). Structure de la gonade femelle chez quelques espèces du genre *Xiphinema* (Nematoda Dorylaimidae) — *Nematologica*, **6**, 144-154.

Luc, M. & de Guiran, G. (1960). Les nématodes associés aux plantes de l'Ouest Africain. Liste préliminaire — *Agron Trop.*, **15**, 434-449.

Luc, M. & Hoestra, M. (1960). Les nématodes phytoparasites des sols de cocoteraie du Togo. Essai d'interprétation du peuplement — *Agron. Trop.* **15**, 497-512.

Siddiqi, M. R. (1959). Studies on *Xiphinema* spp. (Nematoda: Dorylaimidae) from Aligarh (North India), with comments on the genus *Longidorus* Micoletzky, 1922 — *Proc. helm. Soc. Wash.*, **26**, 151-163.

Thorne, G. (1937). Notes on free-living and plant-parasitic nematodes. III — *Proc. helm. Soc. Wash.*, **4**, 16-18.

—— (1939). A monograph of the nematodes of the superfamily *Dorylaimidae* — *Cap. Zool.* **8**, part 5, 261 pp.

Williams, J. R. (1959). Studies on the nematode soil fauna of sugar cane fields in Mauritius. 3. Dorylaimidae (Dorylaimoidea, Enoplida). — *Mauritius Sugar Ind. Res. Inst., Occ. Pap.* **3**, 28 pp.

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Nematologica 6 (1961): 123-129. Leiden, E. J. Brill

ON THE EFFECTS OF ROOT DIFFUSATES FROM *TAGETES* SPP. ON *HETERODERA ROSTOCHIENSIS* WOLL.

BY

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The hatching stimulus or nematicidal effect of *Tagetes* root diffusates on potato-root eelworm was investigated.

Root diffusates of potato, tomato and *Solanum melongena* were also used. Plain water was used as a control. Neither hatching power nor nematicidal effects were detected from three species of *Tagetes* root diffusates.

Analysis of covariance suggests that the biological differences between active root diffusates from different sources are most clearly revealed when counts of emerged larvae are expressed in terms of the weight of oven-dried roots. *S. melongena* root diffusate showed a weaker stimulatory power than those of potato and tomato.

Observations by Steiner (1941) and Oostenbrink, Kuiper & 's Jacob (1957) have shown that the cultivation of *Tagetes* appears to suppress the population of some nematodes. It has also been claimed that a population of *Heterodera rostochiensis* is reduced by as much as 80% in the presence of *Tagetes minuta* (Henry Doubleday Research Association 1960 [2])). Although it has been shown that the juice obtained from plants of *Tagetes nana* was ineffective in vitro against cysts of the potato-root eelworm, a preparation extracted from this plant had a high nematicidal activity against several nematodes, including *Heterodera rostochiensis* larvae (Uhlenbroek & Bijloo 1958).

The reduction of the population of *Heterodera rostochiensis* in the presence of *Tagetes* may be due either to a hatching stimulus present in the roots of the plant, inducing the larvae to hatch and die out, or to a nematicidal effect. The present paper records an investigation into cysts exposed to graded dilutions of root diffusates extracted from three species of *Tagetes* and three known hosts: potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* L.) and egg-plant (*Solanum melongena* L.).

MATERIALS AND METHODS

In all experiments, cysts were set up in batches of one hundred in solid watch glasses and presoaked for a week in an incubator at 25°C. The water was then removed and replaced by the appropriate sample of root diffusate. To minimize errors due to the variability of the cysts, duplicated batches of 100 pot-raised cysts (cysts of known age) and of similar size (599-422 μ) were used in each treatment.

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1) At present on study leave from the University of Agriculture, Tehran, Iran.

2) Interim report on the control of potato eelworm.

Root diffusate collection

Seeds of three species of *Tagetes* (*T. minuta, T. florida, T. signata*) and of eggplant (*Solanum melongena,* long purple) and tomato (Leader) were planted in seed boxes and then transferred into 5 cm diam. clay pots, with a non-sterilized soil mixture of three parts loam to one part sand. Small tubers of potato (Arran Banner) were also planted in 5 cm diam. clay pots. Five pots of each plant were placed on the concave grooves of a sloping asbestos sheet and root leachings were collected by the Fenwick method (1949), i.e. after saturating the soil with tap water, 40 ml of excess water were added, and collected in a jar at the end of the asbestos sheet.

Leaching was carried out twice a week for a fortnight. The entire collection of root diffusate from all the leachings was stored in bottles, and kept in a refrigerator at 5°C.

Because of the suggestion that the most active root diffusate is produced in the early stages of growth (Fenwick 1941), the plants chosen for root diffusate were about 6-10 weeks old, and leachings were collected only 4 times at 3 day intervals.

Weights of haulms and roots

To relate the efficacy of the leachings of each plant to the weight of the haulms and the weight of the roots as well as the age of the plants, after taking the fourth root diffusate the haulms of the plants were removed and the roots were carefully washed and weighed. Then both were placed in an oven at 105°C and dried to constant weight. The results are shown in Table I.

TABLE I

Plant weights per 5 plants (gm)

Plant	Age when lifted	Fresh		Oven dried	
		Roots	Haulms	Roots	Haulms
Eggplant	16 weeks	17.500	81.100	1.390	8.200
Potato	6 weeks	26.120	65.100	2.490	7.185
Tomato	10 weeks	32.500	109.000	2.240	9.750
T. minuta	9 weeks	27.550	24.610	2.220	4.275
T. signata	8 weeks	10.320	23.700	0.870	2.720

Thus, the numbers of larvae hatching in root diffusate in a given time have been expressed in three ways: (a) the actual number counted, (b) the number per g of fresh root and (c) the number per g of dry root.

Setting up: sub-culturing

Collected root diffusates were set up in a graded geometric series of dilutions, the dilutions being 1/1, 1/10 and 1/100.

Duplicate batches of 100 cysts were exposed to the action of each dilution.

The larval emergence for each dilution was estimated every 8 days, up to a period of 48 days at which time emerging larvae in all dilutions were not greater than in tap water. Hatching tests were carried out at 25°C constant temperature. Tap water was used as a control. The results of the tests are expressed as total numbers of liberated larvae per 200 cysts and are shown in Table II.

TABLE II

The number of emerged larvae per 200 cysts from six root diffusates and water

Plant	Number of emerged larvae from 200 cysts		
	Concentration 1/1	Concentration 1/10	Concentration 1/100
Tagetes minuta	829	954	1041
Tagetes signata	735	1075	873
Tagetes florida	1159	777	958
Water	1079	991	808
Eggplant	20526	10506	3113
Potato	54475	36119	19517
Tomato	60038	43743	24343

RESULTS

Hatching power of Tagetes *plants*

An analysis of variance of the *Tagetes* data showed that there was no significant difference between the diffusates of the different species and plain water; but there was a possibility that owing to nematicidal effect, some larvae of the cysts exposed to the root diffusate of *Tagetes* plants were killed, and survivors which hatched happened to be equal to the hatch in water.

To deal with this possibility the batches of 100 cysts remaining after exposure to the leachings of *Tagetes minuta* and water were placed in potato root diffusate. The emerging larvae in each treatment were counted at 8 day intervals. The results are shown in Table III: there was no evidence of delayed hatching and no trace of nematicidal effect.

TABLE III

The number of emerged larvae from batches of 200 cysts already exposed to Tagetes minuta *root diffusate and water for 48 days and then immersed in ordinary root diffusate*

Root Diffusate	Concentrations	Total hatch in ordinary R.D.
Tagetes minuta	1/1	43589
	1/10	43334
	1/100	45141
Tap water	...	43754

Hatching power of the different hosts

Table IV shows the analysis of hatching data in response to the three active diffusates, using a logarithmic transformation. Similar analyses have also been

TABLE IV

Analysis of variance for data of Table II. Tomato, Potato, Eggplant (uncorrected). Log (emerged larvae)

Sources	Degrees of freedom	Sum of Squares	Mean Squares	Variance Ratio
a Concentrations	2	0.950	0.475	a/c 12.8*
b Varieties	2	1.518	0.759	b/c 20.5**
c Interaction	4	0.148	0.037	c/d 10.5*
d Error	9	0.032	0.0035	
e Total	17	2.648		

* = Significant at the 5% level of probability
** = Significant at the 1% level of probability

made for the same counts expressed per gramme of fresh roots and per gramme of dry roots. From these three analyses (the antilog of the treatment means being geometric means) the data of Table V have been computed. These data are presented graphically in Fig. 1 (1-3), which follows the technique of Fenwick (1952) with the difference that the dilution ratio was 10 instead of 4. The values of *b* shown are the decrease in larvae emerging from 100 cysts for each 10-fold increase in dilution.

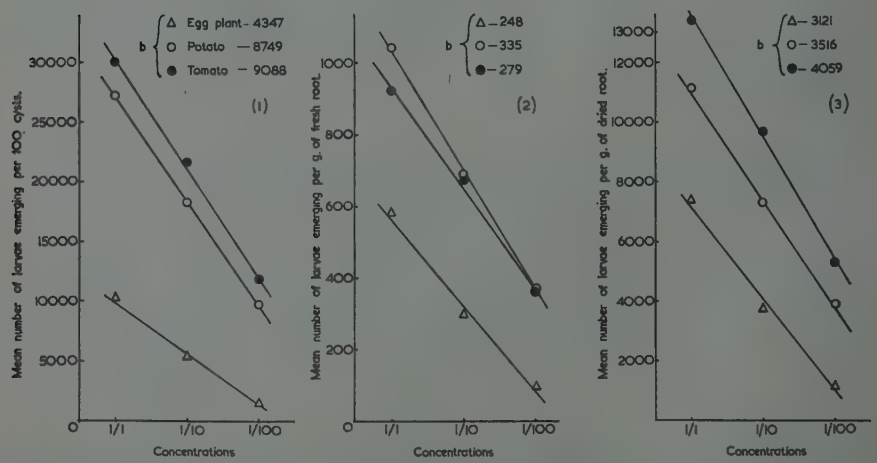

Fig. 1. Emergence of larvae from batches of 100 cysts, (1) uncorrected data, (2) per g of fresh root, (3) per g of dried root.

TABLE V

Number of emerged larvae (Geometric means)

	Plant	Uncorrected data		Data per g fresh root		Data per g oven dried root	
		Geometric Means	Ratio of Means	Geometric Means	Ratio of Means	Geometric Means	Ratio of Means
I	Tomato	19770		609		8831	
			I/II				
			1.175$^{n.s.}$		0.946$^{n.s.}$		1.306*
II	Potato	16827		644		6761	
			II/III				
			3.855*		2.586*		2.152*
III	Eggplant	4395		249		3141	
Concentration							
IV	1/1	20777		826		10257	
			IV/V				
			1.596*		1.601*		1.595*
V	1/10	17706		516		6427	
			V/VI				
			2.254*		2.243*		2.254*
VI	1/100	5636		230		2851	
		Critical ratio 1.194		Critical ratio 1.192		Critical ratio 1.194	

$^{n.s.}$ = not significant
* = significant at the 5 % level of probability

DISCUSSION

The three sets of data: (I) uncorrected hatched larvae for each root diffusate (Table IV and V), (II) the number of hatched larvae per gramme of fresh root and (III) per gramme of oven dried root, were analysed, results being shown in Table V. In uncorrected counts and counts per g of fresh root, there was no significant difference between the means for tomato and potato root diffusates but there was a significant difference between those of potato and eggplant. In counts per g of dried root the differences between tomato, potato and eggplant were all significant. The slope of each line was calculated and compared in three ways (Fig. 1).

The slopes of the three lines for dried root counts do not differ significantly, whereas for the uncorrected counts there is a strong suggestion of a shallower slope for eggplant diffusate. Thus, the dried root counts are more clearly in agreement with the hypothesis put forward by Fenwick (1952), namely, that root dif-

fusates from different sources give parallel slopes. It is also interesting that sensibly straight lines, as found by Fenwick, are given by all the present data.

Thus, in comparing diffusates from different sources there would seem to be a case for expressing counts of emerged larvae in terms of the weight of oven dried roots.

Fassuliotis & Feldmesser (1954) reported that the rates of hatching in root diffusates of eggplant and potato did not differ significantly. But after many different experiments some of which have been described in this paper, I am unable to confirm this result. Possibly it was due to using different strains of *Heterodera rostochiensis* cysts. Other experiments with another strain of cysts have revealed that in three kinds of assays (hatching test, penetration of larvae into the root and final cysts density assay) the eggplant was a host inferior to tomato and potato, and this is confirmed by the present experiment.

Uhlenbroek & Bijloo (1958) reported that some highly nematicidal polythienyls have been isolated from the roots of *Tagetes* plants and one of the compounds was shown to be α-terthienyl. After long extraction and purification processes only 0.2 g of pure α-terthienyl was obtained from 24 kg of *Tagetes* roots. This suggests that the amount of nematicide present in *Tagetes* roots is so small that in short-term pot experiments it is negligible. There is also other evidence (Oostenbrink, Kuiper & s'Jacob 1957) that cultivation of *Tagetes* for a short period either in spring or autumn had no effect on reduction of the nematodes present in the soil, whereas a period of 3-4 months cultivation was effective. Thus it may be that the very small production of nematicide requires a long period of time in order to reveal itself.

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RÉSUMÉ

Effets des secrétions radiculaires de Tagetes *spp. sur* Heterodera rostochiensis *Woll.*

Differentes expériences ont été entreprises afin d'étudier l'action stimulante ou l'effet nématocide de la secrétion radiculaire du genre *Tagetes* sur les nématodes dorés de la pomme de terre. Les secrétions radiculaires de trois hôtes ont été utilisées. L'eau pure a été utilisée comme témoin. Les secrétions radiculaires de trois espèces de *Tagetes* ne possèdent ni le pouvoir de faire éclore les larves ni des effets nématocides. L'analyse de covariance semble montrer que les différences biologiques entre les secrétions actives obtenues de différentes sources sont clairement démontrées quand le nombre de larves écloses est exprimé en fonction du poids des racines séchées à 105° C. Des secrétions radiculaires d'aubergine ont montré une plus faible stimulation que celles de la pomme de terre et de la tomate.

REFERENCES

Fassuliotis, G. B. & Feldmesser, J. (1954). Infection of eggplant, *Solanum melongena*, by the Golden nematode of potatoes *Heterodera rostochiensis*. *Plant Dis. Reptr* **38**, 791-793.

Fenwick, D. W. (1949). Investigations on the emergence of larvae from the cysts of the potato root eelworm *Heterodera rostochiensis* (Woll.) I. *J. Helm.* **23**, 157-170.

—— (1952). The bio-assay of potato root diffusate. *Ann. appl. Biol.* **39**, 457-467.

Fenwick, D. W. & Widdowson, E. (1958). The conduct of hatching tests on cysts of the potato root eelworm *Heterodera rostochiensis* (Woll.) *J. Helm.* **32**, 125-134.

OOSTENBRINK, M., KUIPER, K. & s'JACOB, J. J. (1957). *Tagetes* als Feindpflanzen von *Pratylenchus* Arten. *Nematologica* **2**, 424-433.

UHLENBROEK, J. H. & BIJLOO, J. D. (1958). Investigations on nematicides. I. Isolation and structure of a nematicidal principle occurring in Tagetes roots. *Rec. Trav. chim. Pays-Bas.* **77**, 1004-1009.

—— & —— (1959). Investigations on nematicides. II. Structure of a second nematicidal principle isolated from Tagetes roots. *Rec. Trav. chim. Pays-Bas.* **78**, 382-390.

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PLANT SPECIES ATTACKED BY ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) IN THE FEDERATION OF RHODESIA AND NYASALAND

BY

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SUPPLEMENTARY LIST NO. 2

Previous lists of plants attacked by *Meloidogyne* spp. in the Federation of Rhodesia and Nyasaland, have been published by Martin (1958, 1959). It has been observed that, not infrequently, there is no direct correlation between the degree of galling and the number of mature females and egg masses present. Therefore, as in previous papers, in addition to listing the plants attacked, information is given in column two, on the degree or extent of galling and the abundance of egg masses present.

In all cases, the number of plant specimens examined and the number of different areas or sites from which they were obtained, is shown in column three.

Unless otherwise stated, all observations were made on naturally occurring infections and not from experimental work. The infection rating figures given are the maximum ones recorded, but where values are less than six, it does not necessarily mean that higher values cannot be obtained.

Second column

Figures not enclosed by brackets

0 = No galls visible to the naked eye
1 = Only one or two small swellings present
2 = Few swellings present
3 = Swellings easily found
4 = Swellings visible at a glance
5 = Numerous swellings present
6 = Roots almost totally covered with swellings

Figures enclosed by brackets

(0) = No egg masses seen
(1) = Only one or two egg masses present
(2) = Few egg masses present
(3) = Egg masses readily found
(4) = Egg masses visible at a glance
(5) = Egg masses numerous

acr. = *Meloidogyne incognita* var. *acrita*
ar. = *M. arenaria*
hap. = *M. hapla*
jav. = *M. javanica*

Third column

A = Single plant examined.
B = Two to five plants examined.
C = Six to ten plants examined.
D = Eleven to twenty plants examined.
E = More than twenty plants examined.
Numbers 1, 2, 3 etc. indicate the number of different infested sites from which plant specimens were obtained.

Scientific, common and varietal names of plants	*Meloidogyne* spp. with response and host values	No. of observations and sites
Acacia dealbata Link, Silver wattle	jav. 3	A. 1
Acacia decurrens Willd., Green wattle	jav. 5	A. 1
Acacia elata A. Cunn	jav. 4	A. 1
Acacia mollissima Willd., Black wattle	ar. 6	E. 6
Achyranthes aspera L..	ar. 4 (2)	B. 2
Acrocephalus callianthus Briq.	jav. 3	A. 1
Aeolanthus gamwelliae G. Tayl.	jav. 5	A. 1
Albuca melleri Baker	jav. 1	A. 1
Allium cepa L., Onion, var. Texas Grand	acr. 2	A. 1
Allium porrum L., Leek	acr. 3	A. 2
Ananas comosus (L.) Merr., Pineapple	jav. 3	D. 7
Anthospermum hispidulum E. Mey.	hap. 1	A. 1
Apium dulce Mill. var. *rapaceum* (Mill) DC., celeriac	ar. 4	A. 1
Arachis hypogaea L., Groundnut, var. Red Valencia	hap. 3	E. 1
Aster sp., Michaelmas Daisy	ar. 2	A. 1
Bauhinia variegata L.	jav. 4	B. 1
Beta vulgaris L. var. *cicla* Swiss Chard, var. Lucullus	acr. 4	B. 1
Bidens biternata (Lour.) Merr. & Sherff, Blackjack	hap. 4	B. 1
Bidens pilosa L., Blackjack	jav. 4	E. 1
Bidens sp.	ar. 4 (2) hap. 4 jav. 2	B. 1
Bidens steppia (Steetz) Sherff	jav. 3	C. 3
Borreria diodon K. Schum.	ar. 3	B. 2
Brassica oleracea var. *capitata*, Cabbage, var. Charleton Wakefield	acr. 3	A. 1
Id. var Copenhagen	jav. 4 (2)	A. 1
Id. var. Flat Dutch	jav. 2	A. 1
Brassica rapa L., Turnip, var. Purple Top	ar. 2	B. 1
Buchnera sp.	hap. 1	A. 1
Canna sp.	ar. 2 (0)	A. 1
Capsicum frutescens L., Chili Peppers	acr. 4, jav. 2	B. 3
Id. var. Californian Giant	acr. 4	E. 1
Carica papaya L., Pawpaw Melon, var. Hawaiian Solo	ar. 3 (2) jav. 6	B. 1
Chenopodium album L., Goosefoot	jav. 4	B. 1
Chrysanthemum frutescens L.	hap. 5 (2)	A. 1
Coleus barbatus Benth.	ar. 6	B. 1
Coprosma sp., variegated	jav. 4	B. 1
Corchorus tridens L.	acr. 6	B. 2
Crotalaria ochroleuca G. Don, var. Philipsii	acr. 2 (0), jav. 2 (1)	E. 1
Cucumis sativus L. Cucumber	acr. 4 (2)	A. 1
Id. var. Colorado	acr. 5	A. 1
Cucumis sp. (uncultivated)	jav. 1	A. 1
Cucurbita pepo L., var. *medullosa* Alef., Marrow, var. Ironmark	acr. 5 (3)	A. 1
Curcuma sp.	jav. 4	A. 1
Cyathula cylindrica Moq.	jav. 3	A. 1
Dahlia pinnata Cav., 3 vars.	jav. 6	E. 1
Id. var Golden Leader	jav. 3	B. 1
Daucus carota L., Carrot, var. Imperator	acr. 3	A. 1
Dioscorea bulbifera L.	ar. 5 (0)	A. 1

Scientific, common and varietal names of plants	*Meloidogyne* spp. with response and host values	No. of observations and sites
Eleusine africana Kennedy O'Byrne, Rapoko Grass	acr. 3, ar. 2 jav. 3	acr. B. 1 ar. B. 1 jav. E. 5
Eragrostis racemosa (Thunb.) Steud, Miami Love grass	ar. 2 (0)	A. 1
Euonymus japonicus L. var. *albo-marginatus* Rehd.	jav. 2	B. 1
Euphorbia hirta L.	jav. 3	A. 1
Fragaria X *ananassa* Duchesne, Strawberry, Cultivated hybrids	jav. 2	E. 1
Furcraea gigantea Vent., Mauritius Hemp	jav. 3	A. 1
Geranium simense Hocht. ex A. Rich	hap. 1	A. 1
Gladiolus hybridus Hort., var. Princess Beatrice	acr. 3	B. 1
Gomphrena celosioides Mart.	ar. 3	A. 1
Gossypium hirsutum L., Upland Cotton, var. M.D.L.C.L. 20	ar. 1	E. 1
Halleria lucida L.	jav. 3	A. 1
Helichrysum setosum Harv.	hap. 3	A. 1
Helichrysum umbraculigerum Less.	hap. 2	A. 1
Hemerocallis hybrid	jav. 3 (2)	B. 1
Hibiscus meeusei Exell	jav. 3	A. 1
Hibiscus rosa-sinensis L., Trade var. miniatus	jav. 4	C. 1
Hibiscus schizopetalus Hook.	ar. 4 (2)	B. 1
Holmskioldia speciosa Hutch & Corbishley	jav. 2	B. 1
Hoya carnosa R. Br., Wax flower	jav. 3	A. 1
Hypericum patulum Thunb.	hap. 4	C. 1
Impatiens sp., near *I. sultana* Hook, f. & *I. holstii* Eng. & Warb.	ar. 2 jav. 2	B. 1
Indigofera subulata Vahl ex Poir	jav. 3	A. 1
Iochroma tubulosum Benth.	jav. 4	B. 2
Kalanchoe marmorata Bak.	ar. 4	B. 2
Lagenaria siceraria (Molina) Standl.	ar. 5 (2)	A. 1
Lathyrus odoratus L., Sweetpea	ar. 3	A. 1
Lopholaena coriifolia (Sond.) Phillips & C. A. Smith	jav. 4	A. 1
Lotus corniculatus L., Birdsfoot Trefoil, 2 vars.	jav. 3	E. 1
Id., 3 vars	jav. 4	E. 1
Id., var. Tana, U.S.A. FC 34138	jav. 5	D. 1
Lotus uliginosus Schkuhr, Beaver Big Trefoil, var. U.S.A. FC 32694	jav. 6	D. 1
Lycopersicum esculentum Mill., Tomato, 6 vars.	jav. 5 to 6	E. 6
Id. var. Stoner's Money Maker	acr. 6	E. 2
Id. var. Sutton's Early Market	jav. 2	B. 1
Monsonia biflora DC.	jav. 3	A. 1
Mucuma pruriens (Medik) DC. var. *utilis* (Wall.) Bak. ex Burck.	jav. 4	E. 3
Musa paradisiaca L., Plantain	acr. 3	A. 1
Id. var. Gros Michel	jav. 2	A. 1
Nicotiana rustica L., Snuff tobacco	jav. 2	B. 1
Nicotiana tabacum L., Burley type	jav. 2	A. 1
Panicum atrosanguineum Hochst. ex A. Rich	jav. 2	A. 1
Pelargonium zonale Ait.	ar. 2	A. 1
Petroselinum crispum (Mill.) Airy Shaw, Parsley, var. Moss curled	acr. 3	A. 1
Petunia hybrida Vilm., nana compacta	acr. 5 (3)	A. 1
Phaseolus limensis Macf., Butter Bean	acr. 4	B. 2

Scientific, common and varietal names of plants	*Meloidogyne* spp. with response and host values	No. of observations and sites
Phaseolus vulgaris L., Dwarf Bean, 3 vars.	acr. 3 to 4	C. 4
Id., 4 vars.	jav.. 4 to 5	B. 4
Phytolacca octandra L.	jav. 5 (3)	A. 1
Piper betle L., Betel Pepper Tree	ar. 4 (2)	A. 1
Pisum sativum L. var. *pachylobum* Lam., Field Pea	jav. 6	E. 3
Pisum sativum L. var. *saccharatum* Lam., Garden Pea	acr. 2	B. 1
	hap. 5	
Id., 8 vars	jav. 5 to 6	E. 10
Id. var. Greenfeast	ar. 4	B. 2
Prunus persica (L.) Batsch, Peach	acr. 6	A. 1
Id. Kakumus seedling	jav. 4	A. 1
Raphanus sativus L., Raddish, var. French Breakfast	jav. 2	A. 1
Id. var. Scarlet Turnip	acr. 4	B. 1
Ricinus communis L., Castor Oil Plant, Uncultivated	jav. 2	A. 1
Rosa multiflora Thunb., Rose Rootstocks	hap. 4	E. 2
Rottboellia exaltata L. f.	jav. 4 (0)	A. 1
Rubus idaeus L.	jav. 4 (0)	A. 1
Rumex acetosella L.	ar. 1	B. 1
	jav. 1	
Saccharum officinarum L., Sugar Cane	ar. 3	B. 2
	jav. 5	
Senecio tamoides DC., Canary Creeper	jav. 2	A. 1
Sesamum indicum L.	jav. 3	B. 1
Sesbania caerulescens Harms	jav. 3	A. 1
Sinningia speciosa Benth. & Hook., Gloxinia	ar. 4	A. 1
Solanum mammosum L., Pigs' Ear Plant	acr. 5	A. 1
Solanum melongena L. var. *esculentum* var. Fort Myers	acr. 5	A. 1
Solanum nigrum L., Black Nightshade	hap. 5	A. 1
Solanum seaforthianum Andr.	jav. 4	B. 1
Solanum tuberosum L., Potato, var. Roslin Sasumua	jav. 4	C. 1
Id., var. Walanga	acr. 3	E 1
Solanum sp., near *S. panduriforme* E. Mey	jav. 6	B. 2
Spiraea cantoniensis Lour. var. *lanceata* Zabel	jav. 5	A. 1
Symphoricarpos rivularis Suksdorf	hap. 3	B. 1
Thea sinensis L. (*Camellia sinensis* (L.) Kuntze.) Tea	acr. 3 (1)	E. 1
Theobroma cacao L., Cocoa	jav. 3	B. 1
Thunbergia erecta (Benth.) Anders.	jav. 3	A. 1
Tithonia rotundifolia (Mill.) Blake	jav. 3	A. 1
Trifolium alexandrinum L., Burseem Clover, var. U.S.A. FC 34284	jav. 6	E. 1
Trifolium burchellianum Ser. var. *johnstonii* (Oliv.) Gillett	hap. 5 (3)	E. 1
Trifolium hirtum All., Rose Clover, var. U.S.A. FC 24960	jav. 6	E. 1
Trifolium hybridum L., Common Alsike Clover, var. U.S.A. FC 32587	jav. 6	E. 1
Id. var. U.S.A. FC 34088	jav. 6	E. 1
Trifolium incarnatum L., Crimson Clover, var. Chief Crimson, U.S.A. FC 34234	jav. 6	E. 1
Id. var. Dixie Crimson, U.S.A. FC 34235	jav. 6	E. 1
Trifolium lappaceum L., Burr Clover, var. U.S.A. FC 24196	jav. 6	E. 1
Trifolium masaiense Gillett, Kenya Clover	jav. 6	E. 1

Scientific, common and varietal names of plants	*Meloidogyne* spp. with response and host values	No. of observations and sites
Trifolium pratense L., Red Clover, 71 vars.	jav. 6	E. 1
Trifolium repens L., White Clover, 8 vars.	jav. 6	E. 1
Trifolium rueppellianum Fresen., small type	jav. 6	E. 1
Trifolium resupinatum L., Annual Strawberry Clover	jav. 6	E. 1
Trifolium subterraneum L., Subterranean Clover, var. Tallarock	jav. 6	E. 1
Trifolium usambarense Taub.	jav. 6	E. 1
Triumfetta dekindtiana Engl.	jav. 5	E. 1
Vernonia perrottetii Sch. Bip.	jav. 5	A. 1
Vicia faba L., Broad Bean	ar. 5 (2)	A. 1
Vigna vexillata (L.) Benth..	jav. 2	B. 1
Walafrida swynnertonii S. Moore var. *leiophylla* Brenan	hap. 3 jav. 2	A. 1
Zea mays L., Maize, White Dent type, var. Hickory King	acr. 3 (2)	A. 1
Id., var. Hybrid No. 10, S.E.S.	ar. 3	E. 1
Zea mays L. var. *rugosa* Bonaf., Sweet Corn, var. Stowell's Evergreen.	acr. 3	A. 1
Zinnia elegans Jacq., var. Californian Giant	hap. 3	E. 1

REFERENCES

MARTIN, G. C. (1958). Plant species attacked by root-knot nematodes (*Meloidogyne* spp.) in the Federation of Rhodesia and Nyasaland. *Nematologica* **3**, 332-349.

—— (1959). Plant species attacked by root-knot nematodes (*Meloidogyne* spp.) in the Federation of Rhodesia and Nyasaland. *Nematologica* **4**, 122-125.

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PARASITISM AND PATHOGENESIS OF THE CULTIVATED CRANBERRY BY SOME NEMATODES [1])

BY

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The parasitism of cranberry roots (*Vaccinium macrocarpon* Ait.) by *Hemicycliophora similis* Thorne, *Trichodorus christiei* Allen and *Tylenchorhynchus claytoni* Steiner is described. The symptoms associated with the feeding of these nematodes and *Tetylenchus joctus* Thorne are given. *T. christiei*, which appears to be the most important nematode parasite of cranberry, caused a significant reduction in growth of cuttings and seedlings. The feeding of *H. similis* caused a consistent reduction in the growth of seedlings, but the growth of potted cuttings was not greatly diminished.

Until recently little was known of the stylet-bearing nematodes from the soils of the cultivated cranberry (*Vaccinium macrocarpon* Ait.). Cobb (1913) and Chitwood & Tarjan (1957) collected *Atylenchus decalineatus* Cobb from New Jersey cranberry bogs. Zuckerman & Coughlin (1960) surveyed the cranberry bogs of Massachusetts and recorded styled-bearing nematodes from 24 genera. Except for Zuckerman (1960), who reported parasitism of cranberry roots by *Tetylenchus joctus* Thorne, none gave proof of parasitism or pathogenicity of nematodes to the cranberry. This paper gives the results of experiments which prove parasitism of the cranberry by several stylet-bearing nematodes and describes the associated symptoms.

MATERIALS AND METHODS

Nematode feeding was studied by placing two to sixteen nematodes in Petri plates which contained 4-8 day old cranberry seedlings grown from seed in a 1% water agar medium. The roots of these seedlings frequently grew tightly appressed to the bottoms of the Petri plates. When the plate was inverted feeding was observed up to 200 magnifications under the 10 × objective lens of a compound microscope. Closer observation was attained by two methods. In the first, nematodes were placed in the area of a root and then a cover slip set on the surface of the agar directly over the root. The plate was then inverted and placed in a darkened chamber. The roots, which grew down to the cover slip, could be examined under the high power dry and oil immersion lenses of a compound

[1]) Contribution No. 1286, University of Massachusetts College of Agriculture Experiment Station, Amherst, Mass.

microscope. In the second method a block of agar which contained a seedling and several nematodes was removed from a plate, inverted over a deep well slide and a cover glass placed over the agar. The slide was set in a darkened moist chamber for 24 hours and examined at daily intervals thereafter. These methods allowed the observation of feeding at magnifications up to 1940 ×.

The symptoms associated with feeding were studied by comparing seedlings germinated and grown in (a) soil free of plant parasitic nematodes, (b) soil infested with pure cultures of *Hemicycliophora similis* Thorne or pure cultures of *Trichodorus christiei* Allen, (c) soil taken from a cranberry bog which contained a mixed population of plant parasites or (d) soil which contained no nematodes. The soil was placed in 50 ml beakers and three cranberry seeds placed on the surface in each beaker. Each test was replicated five times and the beakers randomized in a latin square design. The experiment was repeated a second time. The beakers were held in a plastic bag in an incubator at 23°C for 20 days in both experiments, after which the germinated seedlings were removed and their total heights measured and the number of bifurcations in each root counted. Portions of each root system were examined under a microscope. In similar experiments, replicated fifteen times, seedlings grown for 13 days in soil infested with a pure culture of *T. joctus* were compared with seedlings grown in soil free of plant parasitic nematodes.

The symptoms associated with root feeding by *H. similis, T. christiei, T. joctus* and *Tylenchorhynchus claytoni* Steiner were also studied by comparing cuttings grown in soil infested with pure cultures of the nematodes with cuttings grown in soil free of plant parasitic nematodes. The gross effects of feeding by the mixed nematode fauna were studied by growing cuttings in soil to which naturally occurring plant parasitic nematodes from cranberry soils were added. Christie & Perry's (1951) modification of the Baermann technique was used to extract nematodes from soil. Pots were inoculated with 3-64 nematodes of one species which had been hand-picked from a mixed population or about 400 plant parasitic nematodes which represented a random sampling of a heavily infested cranberry soil. These experiments comprised a total of 99 pots. After 240-270 days, the plants were removed from the pots, washed, and a small portion of the roots taken for microscopic examination. The remainder of each plant was placed in an oven at 65°C for 96 hours, then set in a chamber at 23°C and about 70% relative humidity for 72 hours until they had attained equilibrium weight. The roots were then weighed and the lengths of the vines recorded. The nematodes were removed from the soil by the elutriation method described by Seinhorst (1956) and the relative abundance of each species determined.

The effects of nematode feeding were also observed on cuttings grown in small glass-sided containers, which held 100 cc of soil infested with pure cultures of nematodes or 100 cc of soil free of plant parasitic nematodes. A total of 18 flats, each of which contained two cuttings, comprised three tests: soil which contained no plant parasitic nematodes, soil infested with *T. christiei* or soil infested with

T. joctus. After 60 days the new growth was measured and the dry weights of the roots determined.

RESULTS

Feeding – - Hemicycliophora similis

A female *H. similis* observed near a cranberry root in a Petri plate (Fig. 1, A) fed at one site for three hours, then withdrew its stylet and moved it back and forth for a distance of 3-4 μ several times. The nematode then moved several cells distant and probed an epidermal cell at the rate of 3-5 thrusts per second. During the next 3½ hours the nematode probed a number of epidermal cells and then moved away from the root.

. While the nematode fed it lay outstretched, quiescent, and perpendicular to the root. The only movement which was discerned under 430 magnifications was that of the valve in the median oesophageal bulb. The stylet, which penetrated the root to the depth of 55 μ, appeared to be inserted into a vessel.

Feeding *in vitro* was not observed again even though several hundred *Hemicycliophora* were placed in contact with the roots of seedlings in Petri plates. However, further visual evidence of feeding was obtained when cranberry seeds were germinated and grown for 5-7 days in soil infested with *H. similis*. Frequently 1-5 nematodes were found attached to the roots (Fig. 1, C), held by their stylets which had penetrated deeply into the root tissue. If placed in water and not disturbed for a few minutes, the motion of the valve in their median oesophageal bulb recommenced. Ninety-three larvae and adult females were observed for periods up to 48 hours while feeding. The stylet was inserted on an average about half of its total length into the root, though in one case it was observed that 76% of the stylet was inserted. These observations proved that the nematode can insert its stylet deep enough to penetrate a vessel seated well within the root. Most frequently the nematodes fed near the root tip, though they were often found up to one mm behind the root tip.

The germinating of seeds in *Hemicycliophora*-infested soil was used to demonstrate parasitism of *H. gracilis* Thorne, which also occurs naturally in cranberry soils. Seedlings grown for seven days in soil directly from a bog infested with *H. gracilis* had as many as three nematodes attached to the roots. Though this soil contained nematodes from several other plant parasitic genera, only *H. gracilis* was attached to the roots.

The roots of the cuttings planted in the glass-sided planting boxes grew appressed to the glass and, when the cardboard side was removed, many nematodes were clustered around the roots. It was not possible to utilize sufficiently high magnifications to observe the feeding of the nematodes. When the soil was washed from the roots of cuttings grown in these boxes in 56 days, several hundred *H. similis* were attached to the roots of each cutting. Single rootlets had as many as 16 nematodes attached and feeding on their tips.

Feeding — Trichodorus christiei

The feeding of eleven *T. christiei* on roots was observed for varying periods of time, including one series of observations at half hour intervals for 8 consecutive hours. These observations essentially agreed with the detailed description of the feeding of this nematode on the roots of tomato and rye given by Rohde & Jenkins (1957). On cranberry feeding was restricted to the epidermal cells. While feeding the nematode was quiescent and frequently assumed a position roughly resembling the shape of a question mark, with the head perpendicular and in contact with the root (Fig. 1, B). The expansion and contraction of the basal bulb of the oesophagus was readily visible. At the inception of feeding the stylet was rapidly moved in and out at the rate of 5-6 thrusts per second, penetrating the cell to a depth of 3-4 μ. After 30 seconds the rate of thrust slowed to one per second. The protoplasm of the cell shrank rapidly from the cell wall and, coincidentally with the slowing of the rate of stylet thrust, a viscous mass of protoplasm accumulated around the point of entry into the cell by the stylet. When this mass of protoplasm was ingested, the rate of style thrust increased to 5-6 thrusts per second. Feeding on a single cell was of one minute to four minutes and 30 seconds in duration. The nematode then moved to another cell and resumed feeding. As little as ten seconds elapsed between the cessation of feeding on one cell and the initiation of feeding on another. Feeding occurred in all submerged regions of the root, though the nematodes appeared to prefer the area immediately behind the root cap. The walls of the hole made in the cell wall by the stylet penetration appeared coated with a refractive substance, possibly an exometabolite of the nematode. The walls of the hole were visible for several hours after the nematode ceased feeding (Fig. 1, D).

Feeding — Tylenchorhynchus claytoni

Two larvae and one female adult *T. claytoni* were observed feeding on the roots of cranberry seedlings grown in Petri plates. The elapsed time for feeding on one cell varied from two minutes to four minutes and 30 seconds. Prior to feeding the nematode probed the cell several times with its stylet and then inserted it into the cell to a depth of 3-4 μ. While feeding the nematode was quiescent with its head held perpendicular and lips appressed to the cell. The only visible movement was the pulsating of the valvular mechanism of the median oesophageal bulb. Feeding was confined to the epidermal cells and occurred on all portions of the root which were immersed in the agar. The feeding habits for *T. claytoni* on cranberry roots are very similar to those reported by Krusberg (1959) for this nematode on alfalfa roots. Zuckerman (1960) reported that *Tetylenchus joctus* fed on cranberry roots in the same manner.

Root Symptoms

The symptoms associated with nematode feeding on cranberry roots were well-defined, though it is possible that other types of injury may cause similar symp-

B. M. ZUCKERMAN: *Parasitism of Cranberry by Nematodes.*

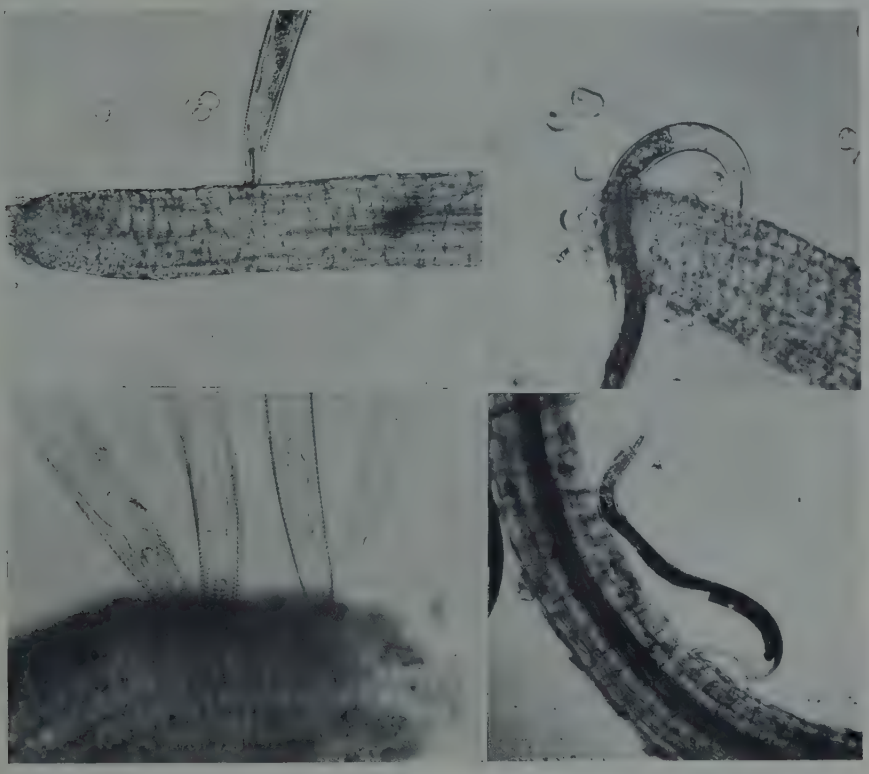


Fig. 1. Nematode feeding on cranberry roots. A) *Hemicycliophora similis* feeding *in vitro*. This nematode has started to withdraw its stylet; prior to this the stylet had penetrated the vascular cylinder (× 175). B) *Trichodorus christiei* feeding near root tip *in vitro* (× 170). C) Four *H. similis* attached to a root tip of a seedling grown in infested soil (× 360). D) *T. christiei* feeding *in vitro* showing the hole made by the stylet in the wall of epidermal cell (× 230).

B. M. ZUCKERMAN: *Parasitism of Cranberry by Nematodes.*

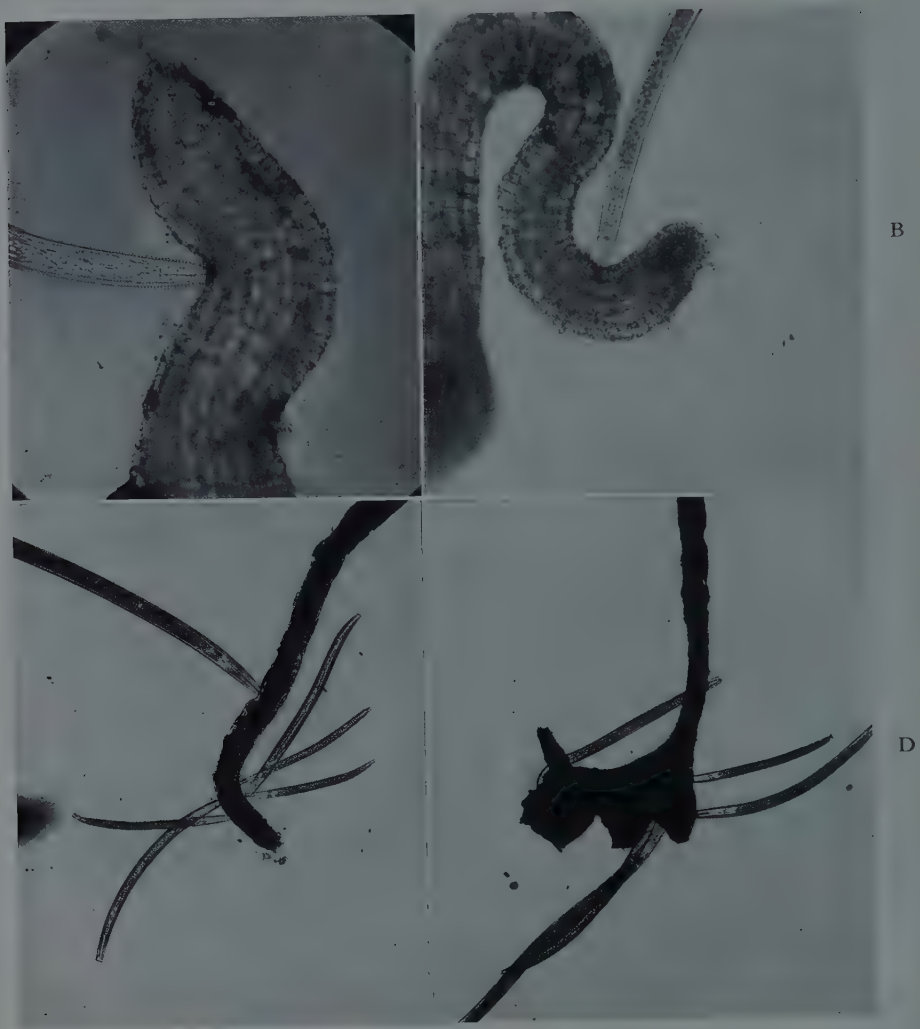
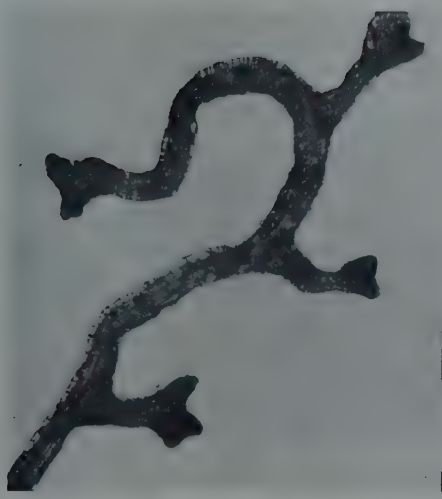


Fig. 2. The symptoms associated with the feeding of *Hemicycliophora similis* on cranberry roots. A) Injury to the cells around the point of penetration of the stylet results in the cessation of growth of these cells (× 200), and B) a distinctive curvature of the root (× 120). C) Frequently many nematodes attack a single root tip (× 50) resulting in D) overgrowth of the root tissues (× 50).

B. M. ZUCKERMAN: *Parasitism of Cranberry by Nematodes.*

A



B

Fig. 3. A) The "stubby root" symptom caused by the feeding of *Trichodorus christiei* on cranberry roots (× 75). B) Collapse of epidermal cells of cranberry root was associated with the feeding of *Tetylenchus joctus* (× 100).

toms. Chandler (1953) stated that root abnormalities in cranberry are often caused by an excess of gravitational water after the new growth has started. Roots on which *H. similis* fed showed two distinct forms of injury, both of which reflect some alteration of the normal growth processes of the root. Cells contiguous to the point of penetration of the stylet appeared to have stopped elongating, whereas normal growth continued on the side of the root opposite stylet penetration (Figs. 2A, 2B). This resulted in a curvature of the root. The second symptom was an overgrowth, or galling, of root tissue (Fig. 2C, 2D). These galls were visible at ten magnifications and very abundant on roots grown in soil infested by large numbers of *H. similis*. Roots from soils not infested with this nematode did not exhibit these symptoms.

T. christiei produced a symptom syndrome on cranberry roots similar to that produced by this nematode on tomato (Rohde & Jenkins, 1957). Lateral roots were aborted and, on some plants, the roots appeared to have proliferated abnormally. While visual comparison indicated that overproliferation of roots was associated with the feeding of *T. christiei,* data gathered in these studies did not support this observation (Table I). The root tips were frequently swollen and gave evidence that one or more adventitious root laterals had undergone an ephemeral period of growth (Fig. 3A).

A collapse of some epidermal cells was associated with the feeding of *T. joctus* and *T. claytoni* (Fig. 3, B). It appeared that this was related either directly to the effects of feeding or to the subsequent entry of fungi into the host cell through the damaged cell wall. Roots grown in soil free of plant parasitic nematodes for comparable periods of time did not exhibit this symptom. Roots from nematode-infested soil appeared to contain more fungus-infested epidermal cells than did roots from nematode-free soil; however, no quantitative data was gathered to support this observation. Roots exposed to nematodes generally were darker than roots from nematode-free soil.

Effects on growth of seedlings

Seedlings grown in soil infested with either *H. similis* or *T. christiei* were significantly smaller than seedlings which had been grown in soil free of plant parasitic nematodes (Table I). Seedlings grown in soil from a heavily infested bog were noticeably smaller than those from nematode-free soil, though these differences were not as consistent as those in the tests referred to above. An infestation of *T. joctus* in the soil did not appear to effect the growth of the seedlings. None of the treatments had a significant effect on the number of branches formed by the seedling roots.

Soil Infestation Studies

The roots of cuttings grown in soil infested with *T. christiei* were significantly smaller than those grown in soil free of plant parasitic nematodes (Table II). Inhibition of root growth occurred whenever large numbers of *T. christiei* were

present. In the experiment of 250 days duration where the population of *T. christiei* was relatively small there was no significant reduction in root growth. *H. similis* may have caused some reduction in root growth, however this was not satisfactorily demonstrated by these experiments. The relatively light infesta-

TABLE I

The effect of plant parasitic nematodes on the growth of cranberry seedlings

Treatment	Duration (days)	Av. Total Length (mm)	Av. # bifurcations/root	Parasitic Nematodes per cc soil
Soil inoculated with *Hemicycliophora similis*	20	18.4**	1.7	71.5
Soil inoculated with *Trichodorus christiei*		16.8**	3.9	45.7
Soil naturally infested with several plant parasitic nematodes		23.5	3.2	50.5
Nematode-free soil		33.2	2.9	0
Soil free of plant parasitic nematodes		37.7	3.4	0
Soil inoculated with *Tetylenchus joctus*	13	24.9	3.3	1.2
Soil free of plant parasitic nematodes		24.4	2.5	0

** significant at the 1% level over plants grown in soil in which on plant parasitic nematodes were present.

tions of *T. joctus* did not affect the quantitative production of roots (Table II). When the soil and plants in the uninoculated series were removed from the pots, the ball of soil was tightly held together by fine, fibrous roots. In contrast, where a significant reduction in root growth occurred, the ball of soil crumbled and did not maintain the shape of the pot. Microscope examination of the roots from the nematode-infested soil revealed an abundance of the symptoms which have been described in this paper as being associated with nematode parasitism. These symptoms were not found on roots of plants grown in nematode-free soil.

DISCUSSION

Van Gundy (1959) reported that the larvae of *Hemicycliophora arenaria* Raski fed only in the region of the root cap. Though *H. similis* was most frequently observed feeding in this area, numerous individuals fed as far as 1 mm behind

TABLE II

Effects of plant parasitic nematodes on the root growth of cranberry cuttings in the greenhouse.

Treatment	Duration (days)	Av. Weight roots (gms.)	Av. Number of plant parasitic nematodes/cc soil at conclusion of experiment			
			T. christiei	*H. similis*	*T. joctus*	*T. claytoni*
Soil inoculated with *Trichodorus christiei*	60	0.013**	6.4	0	0	0
Soil inoculated with *Tetylenchus joctus*		0.160	0	0	1.1	0
Soil free of plant parasitic nematodes		0.165	0	0	0	0
Naturally infested soil from bog	240	0.540**	41.5	28.3	0	3.9
Soil free of plant parasitic nematodes		1.230	0	0	0	0
Soil inoculated with *H. similis* and *T. christiei*	250	0.385	2.1	20.1	0	0
Soil inoculated with *Hemicycliophora similis*		0.444	0	7.5	0	0
Soil free of plant parasitic nematodes		0.514	0	0	0	0
Soil inoculated with *Trichodorus christiei*	270	0.571*	8.8	0	0	0
Soil inoculated with *Tetylenchus joctus*		1.441	0	0	1.7	0
Soil free of plant parasitic nematodes		1.207	0	0	0	0

* Significant at the 5% level over plants grown in soil free of plant parasitic nematodes.
** Significant at the 1% level over plants grown in soil free of plant parasitic nematodes.

the root tip. The thickening of the roots and the formation of corky tissue which characterizes the secondary growth of the roots probably limits the zone of feeding of *H. similis*. These advances in the development of the root structure would prevent the stylet of the nematode from penetrating the vascular tissue. Examination of roots from heavily infested soil revealed some which appeared normal and others which showed symptoms of varying degrees of pathogenicity. If one assumes that the development of the symptoms is dependent on the continued presence and activity of the parasite, it follows that *H. similis* must feed at one site for many days. This hypothesis is supported by the observation that *H. similis* continued feeding for 48 hours on roots which had been removed from the soil and placed in water. It is also feasible that an exometabolite injected into the plant by the nematode prior to feeding might be sufficient in itself to initiate the processes which result in the gall.

These studies indicate that *T. christiei* may be the most important nematode pest of the cranberry. The several species of *Hemicycliophora* which infest cranberry soils may cause a reduction in growth of the cranberry. Seedlings grown in infested soil were significantly reduced in size. Cuttings grown in infested soil over longer periods of time had smaller root systems, but these differences were not statistically significant. This indicates that seeds germinated in infested soil is an extremely sensitive method for demonstrating the effects of nematode feeding. The effects of parasitism of the cranberry by *Hemicycliophora* spp. is the subject of continued investigation.

A survey of 160 cranberry bogs in Massachusetts showed that *Hemicycliophora* spp. infested soil in 72.5% of the bogs and *Trichodorus* spp. infested soil in 41.9% of the bogs (Zuckerman & Coughlin, 1960). This survey proved that these nematodes are endemic in Massachusetts cranberry soils, where they commonly build up in large numbers. Populations of these genera ranged as high as 20 nematodes per cc of soil. *T. joctus, T. claytoni* and nematodes from several other important plant parasitic genera were also found in Massachusetts cranberry soils. The extensive usage of water in cranberry culture probably fosters the development and dissemination of nematodes. It is apparent from these findings that naturally occurring cranberry roots which had not been parasitized to some extent by nematodes are probably non-existent.

RÉSUMÉ

Parasitisme et Pathogénèse des Airelles des marais par des Nématodes.

L'auteur a décrit l'alimentation de *Trichodorus christiei* Allen, *Hemicycliophora similis* Thorne, et *Tylenchorhynchus claytoni* Steiner sur les racines de *Vaccinium macrocarpon* Ait. *in vitro* ainsi que les symptômes associés avec le parasitisme de ces nématodes et de *Tetylenchus joctus* Thorne. *T. christiei*, qui semble être le nématode parasite le plus important de l'hôte, a causé une réduction significative de l'accroissement des racines. *H. similis* a causé une réduction de l'accroissement des racines des jeunes plantes; mais le développement des racines des boutures en pot n'a pas été diminué conséquemment. *T. joctus* et *T. claytoni* n'ont pas inhibé l'accroissement des racines.

LITERATURE CITED

CHANDLER, F. B. (1953). Drainage. *Cranberries* **18**, 17-18.

CHITWOOD, B. G., & TARJAN, A. C. (1957). A redescription of *Atylenchus decalineatus* Cobb, 1913 (Nematoda: Tylenchinae). *Proc. helm. Soc. Wash.* **24**, 48-52.

CHRISTIE, J. R. & PERRY, V. G. (1951). Removing nematodes from soil. *Proc. helm. Soc. Wash.* **18**, 106-108.

COBB, N. A. (1913). New nematode genera found inhabiting fresh water and non-brackish soils. *J. Wash. Acad. Sci.* **3**, 432-444.

KRUSBERG, L. R. (1959). Investigations on the life cycle, reproduction, feeding habits and host range of *Tylenchorhynchus claytoni* Steiner. *Nematologica* **4**, 187-197.

ROHDE, R. A. & JENKINS, W. R. (1957). Host range of a species of *Trichodorus* and its host-parasite relationship on tomatoes. *Phytopathology* **47**, 295-298.

SEINHORST, J. W. (1956). The quantitative extraction of nematodes from soil. *Nematologica* **1**, 249-267.

VAN GUNDY, S. D. (1959). The life history of *Hemicycliophora arenaria* Raski (Nematoda: Criconematidae). *Proc. helm. Soc. Wash.* **26**, 67-72.

ZUCKERMAN, B. M. (1960). Parasitism of cranberry roots by *Tetylenchus joctus* Thorne. *Nematologica* **5**, 253-254.

ZUCKERMAN, B. M. & COUGHLIN, J. (1960). Nematodes associated with some crop plants in Massachusetts. *Bull. Mass. agric. Exp. Sta.* 521.

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Nematologica 6 (1961): 144-154. Leiden, E. J. Brill

STRUCTURE DE LA GONADE FEMELLE CHEZ QUELQUES ESPÈCES DU GENRE *XIPHINEMA* COBB, 1913 (NEMATODA — DORYLAIMOIDEA) [1])

PAR

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L'auteur étudie la structure de la gonade femelle chez 22 espèces du genre *Xiphinema*. Les espèces se distinguent principalement par la plus ou moins grande différenciation des diverses parties de l'oviducte. Une étude détaillée de l'organe Z est donnée; cet organe est maintenant connu chez 2 espèces (*X. ebriense* et *X. ifacolum*). L'atrophie de la gonade antérieure est étudiée chez *X. indicum*, *X. longicaudatum* et *X. ensiculiferum*. La présence de femelles syngoniques est notée chez *X. attorodorum*.

L'observation dans le tractus génital femelle de *Xiphinema ifacolum* Luc, 1961 d'un organe Z encore plus nettement différencié que celui que nous avions décrit chez *X. ebriense* Luc, 1958, nous a conduit à étudier la structure de la gonade femelle chez les espèces du genre *Xiphinema* rencontrées dans l'Ouest Africain et chez quelques espèces américaines et indiennes respectivement transmises par A. C. Tarjan et M. A. Siddiqi que nous tenons à remercier vivement.

Les espèces qui ont été observées sont les suivantes.

X. americanum Cobb, 1913
X. attorodorum Luc, 1961
X. basiri Siddiqi, 1959
X. brasiliense Lordello, 1951
X. brevicaudatum Schuurmans-Stekhoven, 1951
X. campinense Lordello, 1951
X. chambersi Thorne, 1939
X. citri Siddiqi, 1959
X. diversicaudatum (Micoletzky, 1927) Thorne, 1939
X. ebriense Luc, 1958
X. ensiculiferum (Cobb. 1893) Thorne, 1937
X. flagellicaudatum Luc, 1961
X. ifacolum Luc, 1961
X. index Thorne & Allen, 1950
X. indicum Siddiqi, 1959
X. hallei Luc, 1958
X. longicaudatum Luc, 1961
X. longidoroides Luc, 1961
X. nigeriense Luc, 1961
X. radicicola Goodey, 1936
X. setariae Luc, 1958
X. yapoense Luc, 1958.

Cette étude porte sur plus de 150 femelles appartenant à 22 espèces des 32 qui constituent actuellement le genre *Xiphinema* Cobb, 1913.

Le genre *Xiphinema* comprend une grande majorité d'espèces à femelles didelphiques et trois espèces typiquement monodelphiques (*X. chambersi*, *X. radicicola* et *X. brasiliense*) chez lesquelles seule la gonade postérieure est développée. Enfin

[1]) Présenté à la „1ère Conférence Interafricaine de Nématologie", Nairobi, 24-28 Octobre 1960.

quelques espèces caractérisées par la réduction plus ou moins poussée de la gonade antérieure forment transition entre les deux premiers groupes; il s'agit de *X. indicum, X. longicaudatum, X. krugi* Lordello, 1955 et *X. ensiculiferum*. Trois de ces dernières espèces seront étudiées de façon détaillée plus loin.

STRUCTURE GÉNÉRALE DE LA GONADE FEMELLE (fig. 1)

La gonade femelle comprend successivement de sa partie distale à sa partie proximale:

l'ovaire; l'oviducte, avec plusieurs portions différenciées; l'uterus; la vulve.

L'ovaire: (fig. 1a, fig. 2) — L'ovaire est droit, mais dirigé en sens opposé à la vulve; c'est donc à tort que l'on parle chez *Xiphinema* d'un „ovaire réfléchi"; c'est en fait la gonade qui est réfléchie, la flexion se situant dans la portion proximale de l'oviducte. La seule espèce chez laquelle nous ayons observé un ovaire courbé, et encore sur une minorité d'individus seulement, est *X. ebriense*.

L'ovaire est court et trapu; à une partie germigène très brève succèdent des ovocytes formés sur plusieurs rangs, les premiers ayant souvent un aspect en calotte. Ensuite la forme des ovocytes se régularise et ils se trouvent situés en file sur un seul plan; mais le nombre de ces derniers n'est jamais très grand. Il semble qu'à partir d'un certain stade, un seul ovocyte continue son développement jusqu'au stade d'oeuf à membrane bien individualisée, car on n'observe pas tous les stades de transition entre cet oeuf et les jeunes ovocytes. Les oeufs à leur complet développement sont très longs (jusqu'à 305 μ chez *X. chambersi*) dépassant souvent la longueur de l'ovaire; il n'en existe qu'un seul développé par ovaire, et le plus fréquemment chez les espèces didelphiques un seul ovaire contient un oeuf.

La paroi de l'ovaire (figure 2 p) est mince et passe le plus souvent inaperçue; sur certains individus elle peut cependant être observée principalement grâce aux noyaux de ses cellules; il s'agit d'assez grands noyaux (fig. 2 n) de forme souvent irrégulière, et plus granuleux que ceux des ovocytes avec un nucléole moins important.

Chez certaines espèces, dont *X. ensiculiferum, X. indicum* (cf. fig. 15) et *X. ebriense*, la partie germigène est très courte, et les ovocytes s'organisent sur un seul rang beaucoup plus tôt; chez ces espèces l'ovaire est généralement plus long et les différents stades de développement des ovocytes se succèdent avec une discontinuité moins marquée que dans le premier groupe d'espèces.

L'oviducte (fig. 1: b, c, d, e, f, g, h): à l'extrémité distale de l'ovaire, fait suite à la paroi ovarienne une poche à paroi épaisse (fig. 1 b) constituée de cellules allongées transversalement lui conférant un aspect strié très caractéristique; ces cellules peuvent considérablement se distendre surtout dans le sens longitudinal, car c'est très fréquemment que l'on observe un oeuf presque entièrement développé dans cette portion de l'oviducte.

La courbure de l'oviducte se situe après cette structure; lui succède une zone à paroi mince (fig. 1 c), fine, constituée de cellules allongées transversalement,

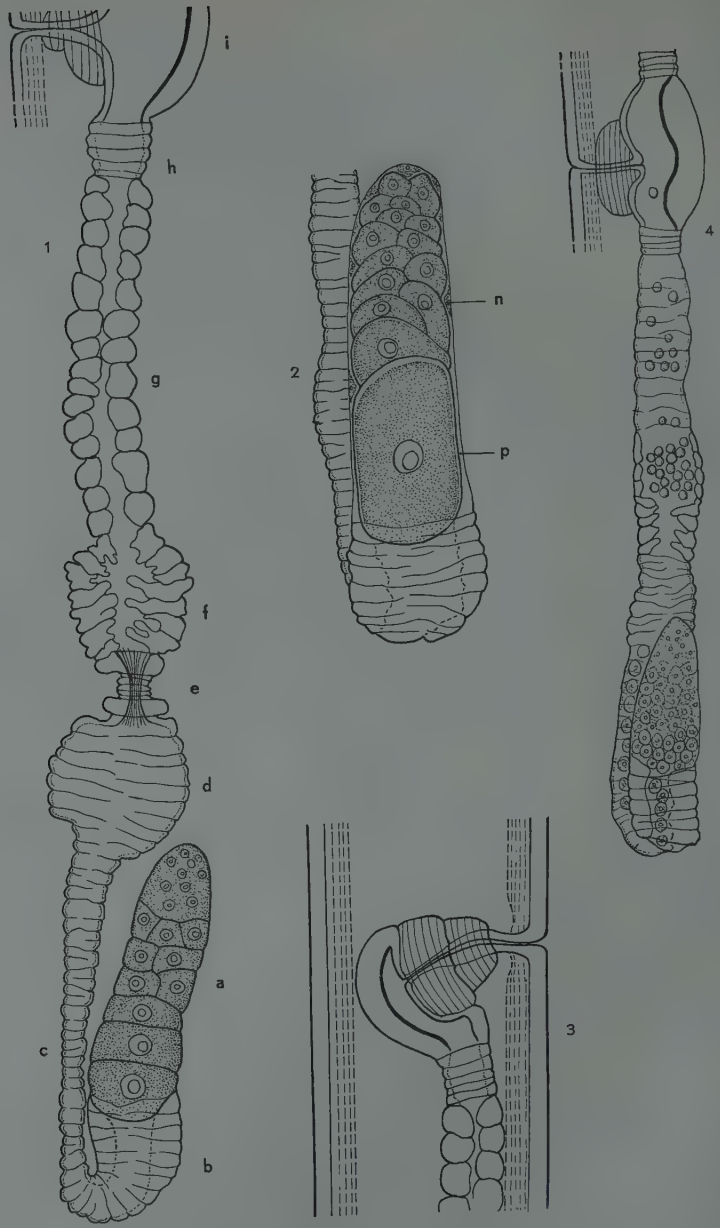

Fig. 1-4. 1: *Xiphinema longicaudatum* Luc, 1961: gonade postérieure. 2: *X. ifacolum* Luc, 1961: ovaire. 3: *X. brasiliense* Lordello, 1951: utérus et vulve. 4: *X. attorodorum* Luc, 1961: femelle syngonique, stade mâle. Gross: 1, 3, 4 × 780; 2, × 1150).

puis une poche (fig. 1 d) généralement située à hauteur de l'extrémité proximale de l'ovaire, poche de structure analogue à la première portion de l'ovaire (fig. 1 b), mais à paroi plus mince; chez les espèces bisexuées c'est très fréquemment à ce niveau que sont stockés la majorité des spermatozoïdes; on peut donc la considérer comme spermathèque, mais peu différenciée.

Cette poche se rétrécit brutalement en une structure musculeuse, le sphincter Z (fig. 1 c): il s'agit d'un organe court, à musculature annulaire puissante; la lumière centrale est bien délimitée et bordée d'une paroi parfois striée longitudinalement; ce sphincter, à sa pleine différenciation, comprend deux bourrelets, l'un apical, l'autre proximal; la structure de cet organe peut d'ailleurs beaucoup varier d'une espèce à l'autre, et même être indiscernable.

Lui succède une poche à cellules d'aspect glandulaire, méandriformes, faisant saillie dans la lumière (fig. 1f), d'une structure toute différente des parties antérieurement décrites. Ensuite vient une portion allongée (fig. 1g) à cellules globuleuses, arrondies; puis une structure à muscles circulaires (fig. 1 h) faisant la jonction avec l'uterus et appartenant peut-être en fait à ce dernier.

L'uterus (fig. 1 i): est bordé d'une paroi musculeuse très épaisse, principalement à la paroi dorsale. Chez les espèces monodelphiques (*X. chambersi, X. brasiliense, X. radicicola*) il n'existe aucun vestige de la gonade antérieure sous forme de sac para-utérin par exemple (fig. 3).

La vulve: est en fente transversale profonde, souvent de la moitié du diamètre correspondant; elle est parfois en croix à grande branche transversale; elle est bordée intérieurement par un repli cuticulaire épais et à son contact avec l'uterus est entourée par un sphincter puissant.

VARIATIONS AUTOUR DE CETTE STRUCTURE

La structure du tractus génital femelle que nous venons de décrire est celle la plus différenciée (mis à part la présence de l'organe Z dont il sera traité plus loin). Les variations autour de cette structure chez différentes espèces affecteront donc surtout la réduction ou l'absence de telle ou telle partie de l'oviducte.

Les variations de la structure de l'ovaire que nous avons déjà notées sont en effet légères et ne concernent que le plus ou moins grand étalement de la zone germigène par rapport à celle des ovocytes, ainsi que l'organisation plus ou moins rapide de ceux-ci sur un seul rang. Encore dans certaines espèces le faible nombre de femelles observées ne permet pas de conclure définitivement s'il s'agit là de deux types différents d'ovaires ou de deux stades différents. *X. ebriense* constitue une exception par certains de ses individus chez lesquels la courbure de la gonade s'effectue au niveau de l'ovaire et non à celui de l'oviducte.

La structure décrite pour l'uterus se retrouve à de très légères variantes près, portant principalement sur la longueur respective des différentes parties et le développement plus ou moins prononcé du sphincter Z, chez les espèces suivantes:

X. brasiliense, X. diversicaudatum, X. hallei, X. longicaudatum (ovaire postérieur), *X. longidoroides, X. nigeriense* et *X. setariae.*

Des structures un peu différentes de ce schéma ont été observées chez les espèces suivantes:

X. americanum: l'ovaire est étroit et allongé; l'uterus très peu différencié; on passe insensiblement de la zone à cellules rondes à celle à cellules parallélipipédiques, sans qu'il y ait trace de sphincter Z ou de poche à cellules sinueuses..

X. attorodorum: la structure de la gonade, très courte, est simplifiée: après l'ovaire, au niveau où l'oviducte se réfléchit, existe une structure analogue à la poche à cellules sinueuses puis l'oviducte se poursuit par une zone à cellules pavimenteuses, sans caractère particulier.

Chez *X. basiri* et *X. campinense* l'oviducte est très allongé, parfois enroulé sur lui-même; la poche à cellules sinueuses est très développée, le sphincter Z petit mais net, à musculature mince, sans bourrelets.

Chez *X. brevicaudatum* l'oviducte est également très long, parfois enroulé sur lui-même mais apparemment sans aucunes parties différenciées.

Chez *X. chambersi, X. radicicola* et *X. yapoense* le sphincter Z est peu développé, très court; la poche à cellules sinueuses est également peu différenciée.

Chez *X. flagellicaudatum* il n'existe pas de sphincter Z et la poche à cellules sinueuses est peu différenciée.

Chez *X. index* l'ovaire est assez allongé; l'oviducte est assez peu différencié: la première poche renflée de l'oviducte est très longue et vient au contact direct de la zone à cellules globuleuses.

X. citri: l'ovaire est sans caractère particulier; les différentes parties de l'oviducte sont très bien différenciées; le sphincter Z („muscular valve" de Siddiqi) est très net, sans bourrelets, sans striations internes, renflé au milieu; il n'existe pas de poche à cellules sinueuses.

L'ORGANE Z

Cette structure que nous avons décrite chez *X. ebriense* a été également rencontrée chez *X. ifacolum.*

Il s'agit d'une portion de l'oviducte occupant la place du sphincter Z (fig. 5a; 6 et 7) et différenciée en un bulbe à musculature circulaire puissante. La lumière de ce bulbe est bordée d'une paroi sclérotisée épaisse, plissée en longueuer, permettant une très efficace dilatation en diamètre. Au centre renflé du bulbe, la lumière est considérablement élargie, et la paroi présente quatre apophyses sclérotisées(ce nombre n'avait pu être précisé dans la description de *X. ebriense,* faite sur individus dans le fixateur) saillant à l'intérieur, de structure simple (fig. 7, 8), peu étroitement accolées les unes aux autres chez *X. ebriense* mais d'aspect assez tourmenté et étroitement engrenées chez *X. ifacolum* (fig. 6, 9) lorsque cet organe est vide.

L'organe Z peut considérablement se dilater au passage des oeufs (comparer les fig. 10 et 11) qui semblent pouvoir y être stockés, un par un, un certain temps.

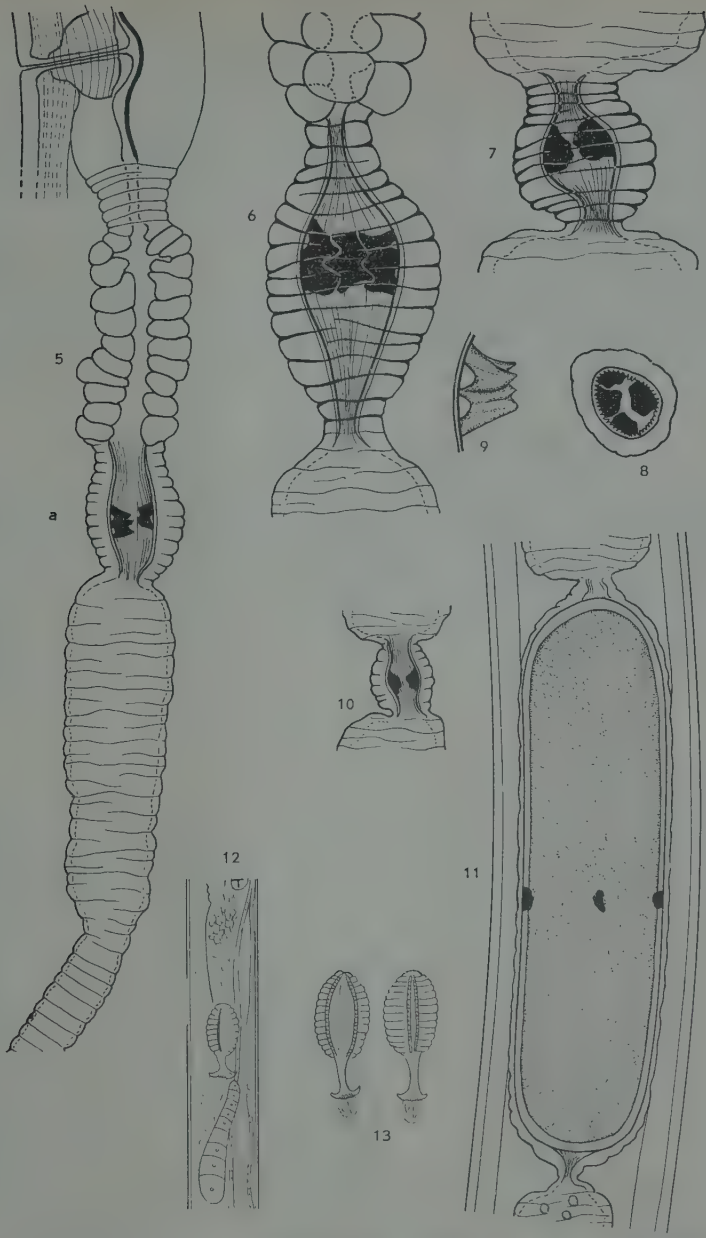

Fig. 5-13. 5, 6, 9 — *Xiphinema ifacolum* Luc 1961; 5: position de l'organe Z (a) dans l'oviducte; 6: organe Z; 9: une apophyse sclérotisée de l'organe Z, en vue latérale. 7, 8, 10, 11 — *Xiphinema ebriense* Luc 1958; 7: organe Z, vue latérale; 8: organe Z vu en bout; 10: organe Z au repos; 11 organe Z contenant un oeuf; 12, 13 — *Heterodorus magnificus* Altherr 1952; 12: gonade femelle postérieure« 13: organe Z. (d'après Altherr). (Gross: fig. 5, 10, 11, × 570 — fig. 6, 7, 8, 9, × 1150).

(Notons que chez *X. ebriense* et *X. ifacolum* il n'existe pas de poche à cellules sinueuses dans l'oviducte).

Le rôle exact de cet organe énigmatique reste inconnu. Une structure semblable a été décrite par Altherr (1952) chez *Heterodorus magnificus* Altherr, 1952. Malheureusement les lames contenant les individus de cette espèce ne sont pas consultables et nous ne pouvons que donner une reproduction des dessins originaux (fig. 12 et 13).

Aucune autre mention d'un organe semblable dans le tractus génital femelle n'a été faite, à notre connaissance, chez les nématodes à l'exception de celle de Rauther (1918) décrivant chez *Macracis monhystera* une structure musculaire d'un type voisin, plus simplifié, mais celle-ci est située à la jonction de l'ovaire et de l'oviducte.

RÉGRESSION DE LA GONADE FEMELLE ANTÉRIEURE

Chez la plupart des espèces didelphiques il est rare que les femelles présentent un développement équivalent des deux gonades, mais c'est tantôt l'ovaire antérieur tantôt le postérieur qui montre le plus grand développement et les différences ne proviennent que du nombre d'ovocytes en fin d'évolution ou de la présence ou l'absence d'oeufs entièrement formés.

Chez quelques espèces cependant la gonade antérieure est constamment moins développée et cette réduction n'affecte pas seulement l'ovaire, lequel peut considérablement régresser et même disparaître, mais également la structure de l'oviducte.

X. indicum représente un premier type:

L'oviducte, long, parfois replié sur lui-même, a le même développement dans les deux gonades; le sphincter Z est très primitif, presque inexistant; la poche à cellules sinueuses est présente mais peu différenciée bien que moyennement développée; l'élargissement de l'oviducte avant le sphincter Z est bien marqué; l'ovaire par contre est extrêmement différent dans l'une et l'autre gonade: l'ovaire de la gonade postérieure est normal (fig. 15); celui de la gonade antérieure (fig. 14) 3 à 4 fois plus court, les ovocytes de la zone germigène sont plus tassés, beaucoup plus petits; ils n'arrivent qu'à un faible développement et ne grossissent pas au-delà de 15 × 7 μ; leur noyau est d'ailleurs beaucoup plus petit que celui des ovocytes de l'ovaire postérieur; de tels ovocytes ne doivent certainement pas arriver à un complet développement et l'ovaire antérieur ne doit pas être fonctionnel.

Chez *X. longicaudatum* la gonade postérieure est tout à fait normale (fig. 1). La gonade antérieure a une structure bien différente (fig. 16): en partant de l'uterus on rencontre d'abord une courte zone à musculature radiale puis directement en connexion avec celle-ci une poche à cellules sinueuses, bien individualisée mais plus petite que celle de la gonade postérieure, un sphincter Z bien reconnaissable puis une file double de cellules sans caractères spéciaux; il n'existe pas d'ovaire antérieur.

Cette structure ressemble en beaucoup de points à celle décrite par Lordello (1955) chez *Xiphinema krugi* Lordello, 1955: la gonade antérieure est apparemment réduite, d'après l'illustration accompagnant l'article, à l'oviducte dans lequel on peut même reconnaître un rétrécissement occupant la place et étant probablement une ébauche de sphincter Z.

Ces espèces présentent donc plusieurs types et stades de régression de la gonade antérieure et montrent le passage vers les espèces typiquement monodelphiques. Chez celles-ci cependant il ne subsiste aucun rudiment de la gonade antérieure (fig. 3).

Chez *X. ensiculiferum* (fig. 17 et 18) la gonade antérieure a la même structure que la gonade postérieure mais elle est réduite dans toutes ses dimensions: l'oviducte n'atteint que la moitié de la longueur de l'oviducte postérieur, de même que l'ovaire; de plus cette réduction affecte également la largeur des organes considérés: alors que le tractus génital postérieur occupe à son endroit le plus large la moitié, ou les trois quarts du diamètre du corps, le tractus génital antérieur n'occupe que le cinquième ou le quart de cette même largeur; de plus ovaire et oviducte sont étroitement accolés l'un à l'autre, rendant l'observation difficile.

Cependant la structure de l'ovaire antérieur semble normale; les noyaux des ovocytes sont de même taille que ceux des ovocytes postérieurs. Le faible nombre de femelles examinées (7) ne permet pas de décider si cette gonade antérieure est fonctionnelle ou non.

HERMAPHRODISE CHEZ X. ATTORODORUM

Sur les neuf individus sexués appartenant à une souche de cette espèce rencontrée au voisinage de racines de *Citrus vulgaris* en Guinée, et légèrement différents des individus typiques, sept possédaient des gonades femelles normales ne différant du schéma général que par la faible différenciation de l'uterus dans lequel on ne peut reconnaître qu'une ébauche de poche à cellules sinueuses et aucune structure correspondant au sphincter Z.

Les deux autres animaux, que la présence d'une vulve nous oblige à nommer également femelles, possédaient un testicule en lieu et place d'un ovaire (fig. 4). En effet au lieu d'observer une zone germinale à noyaux nombreux suivie de cellules en calottes puis d'ovocytes, on observe une partie spermogoniale plus importante, suivie d'un grand nombre de petites cellules se différenciant rapidement en de petits éléments globuleux, très granuleux, qu'il nous faut considérer comme des spermatozoïdes; on peut observer d'ailleurs tous les termes de passage entre les cellules spermogoniales et les spermatozoïdes individualisés.

On observe ces spermatozoïdes dans l'oviducte; ils s'y accumulent principalement dans la portion située en avant de l'ébauche de poche à cellules sinueuses. A ce moment ils sont entièrement développés, ont perdu leur structure granuleuse, sont parfaitement hyalins et arrondis et forment des petites sphères de 1,6-2 μ de diamètre; on en retrouve jusque dans l'utérus.

Nous ne pensons pas que certains animaux jouent le rôle de mâles et d'autres celui de femelles, mais plutôt que le même animal passe par une phase mâle et par une phase femelle. Il s'agirait donc chez *X. attorodorum* d'un cas d'hermaphrodisme successif, vraisemblablement protandre. Les individus observés seraient donc des femelles syngoniques.

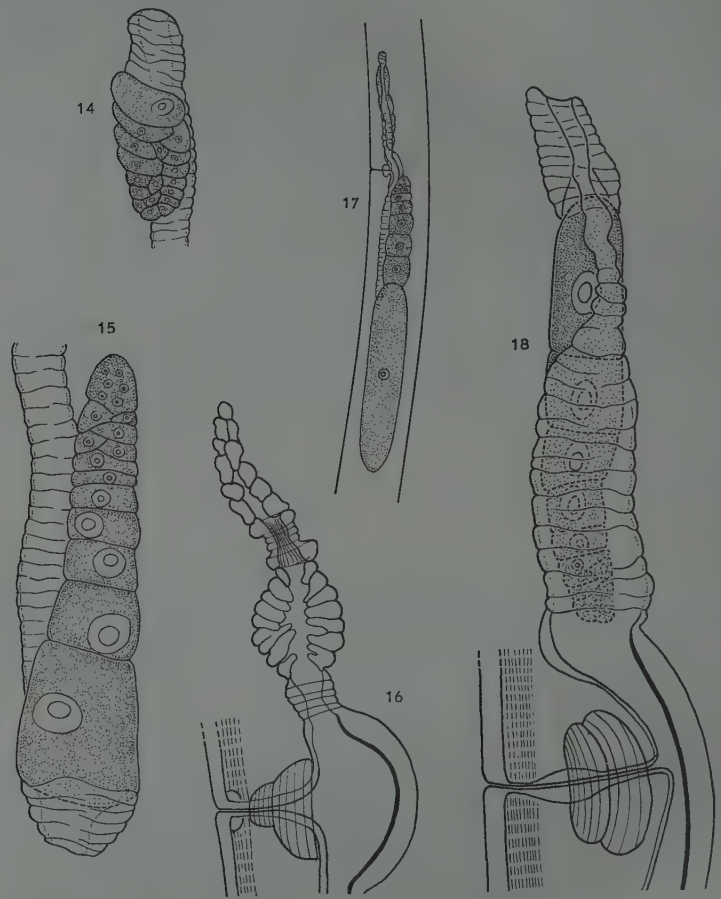

Fig. 14-18. 14-15 — *Xiphinema indicum* Siddiqi, 1959; 14: ovaire antérieur; 15: ovaire postérieur. 16 — *Xiphinema longicaudatum* Luc, 1961; gonade antérieure réduite. 17-18 — *Xiphinema ensiculiferum* (Cobb, 1893) Thorne, 1937; 17: appareil génital femelle; 18: gonade antérieure réduite. (Gross: fig. 14, 15, 16, 18: × 780 — fig. 17: × 250).

Nous n'avons pu noter de façon certaine un fonctionnement analogue des gonades chez les femelles d'aucune des espèces étudiées; cependant chez plusieurs femelles appartenant à *X. longidoroides* et *X. brasiliense,* nous avons observé une

partie spermogoniale à l'extrémité proximale de la gonade et la formation de petits globules à contenu granuleux, tout à fait semblables à des spermatocytes jeunes, venant de se différencier: cependant l'absence de spermatozoïdes nettement différenciés ne permet pas de conclure à l'existence d'un hermaphrodisme chez ces deux espèces.

C'est la première fois, à notre connaissance, qu'un cas d'hermaphrodisme successif est signalé chez les Dorylaimoidea. Il est connu par contre chez d'autres groupes de nématodes: chez *Rhabdias,* parasite des Batraciens et des Reptiles (Schleip, 1911; Schaake, 1931) et, parmi les espèces terrestres, dans différents genres et espèces de Rhabditidae (Maupas, 1900; Potts, 1910; Honda, 1925; Nigon, 1949).

Ce phénomène pourrait expliquer en partie le grand nombre d'espèces chez lesquelles seuls des individus morphologiquement femelles ont été observés.

L'hermaphrodisme simultané, par contre, a été signalé chez *Mononchus ventralis* par Cobb (1918) et chez divers *Helicotylenchus* par Perry (1959).

SUMMARY

Structure of the female gonad in some species of Xiphinema *Cobb, 1913 (Nematoda-Dorylaimoidea).*

The structure of the female gonad was studied in 22 species of *Xiphinema.* The main differences between them were in the degree of differentiation of the various parts of the oviduct. When most fully differentiated the oviduct had the following parts:

— a chamber with muscular thick walls
— a thin walled duct
— a second chamber with muscular walls of medium thickness
— a muscular sphincter (the sphincter Z)
— a chamber lined with sinuous (glandular!) cells
— a duct with four rows of globular cells ending by a sphincter in the uterus.

The variations shown by this structure were studied.

The organ Z, a very complicated sphincter apparatus with internal sclerotised apophyses is described and figured in detail; it occurred in *X. ebriense* and *X. ifacolum.*

The anterior gonad was reduced in three species, representing three different types:

— *X. indicum*: oviduct normal, ovary reduced and not functional
— *X. longicaudatum*: oviduct reduced, no ovary
— *X. ensiculiferum*: oviduct and ovary considerably reduced, not functional.

In *X. attoldorum*, there occur syngonic females with a male phase preceding the female one.

BIBLIOGRAPHIE

Altherr, F. (1952). Les nématodes du Parc National Suisse; Nématodes libres du sol; 2ème partie — *Ergebn. wiss. Unters. Schweiz. NatParc,* **3**, 315-356.

Cobb, N. A. (1918). Nematodes of the low sand filter-beds of American cities — With notes on hermaphroditism and parthenogenesis — *Contrib. Sci. Nem.,* **8**, 189-212.

Honda, H. (1925). Experimental and cytological studies on bisexual and hermaphroditic free-living nematodes, with special reference to problem of sex — *J. Morph.,* **40**, 191-233.

Lordello, L. E. G. (1955). *Xiphinema krugi* n. sp. (Nematoda, Dorylaimidae) from Brasil, with a key to the species of *Xiphinema* — *Proc. helm. Soc. Wash.,* **22**, 16-21.

Luc, M. (1958). *Xiphinema* de l'Ouest Africain: description de cinq nouvelles espèces (Nematoda: Dorylaimidae) — *Nematologica,* **3**, 57-72.

—— (1961). *Xiphinema* de l'Ouest Africain: deuxième note (Nematoda: Dorylaimidae) — *Nematologica*, **6**, 107-122.

Maupas, E. (1900). Modes et formes de reproduction des nématodes — *Arch. Zool. exp. gén.*, sér. 3, **8**, 463-624; pl. 16-26.

Nigon, V. (1949). Modalités de la reproduction et déterminisme du sexe chez quelques nématodes libres — *Ann. Sci. Nat.*, **11**, 1-132; pl. 1-15.

Perry, V. G. (1959). A note on digonic hermaphroditism in spiral nematodes (*Helicotylenchus spp.*) — *Nematologica*, **4**, 87-88.

Potts, F. A. (1910). Notes on the free-living nematodes — *Quart. J. micr. Sci.*, **55**, 433-484; fig. 1-11; tab. 1-2.

Rauther, M. (1918). Mitteilungen zur Nematodenkunde — *Zool. Jb.* **40**, 471-514.

Schaake, M. (1931). Infektionsmodus und Infektionsweg des *Rhabdias bufonis*, und die Metamorphose des genital Apparates der hermaphroditischen Generation — *Z. Parasitenk.*, **3**, 517-648.

Schleip, W. (1911). Das Verhalten des Chromatin bei *Angiostomum nigrovenosum* — *Arch. Zellforsch.*, **7**, 87-183.

Siddiqi, M. R. (1959). Studies on *Xiphinema* spp. (Nematoda: Dorylaimoidea) from Aligarh (North India), with comments on the genus *Longidorus* Micoletzky, 1922 — *Proc. helm. Soc. Wash.*, **26**, 151-163.

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Nematologica 6 (1961): 155-169. Leiden, E. J. Brill

REVISION OF THE HOPLOLAIMINAE (NEMATODA)
I. CLASSIFICATION OF NOMINAL GENERA AND NOMINAL SPECIES [1])

BY

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The genera and species of the subfamily Hoplolaiminae are reviewed and a classification of the genera and species is proposed. The subfamily and genera are diagnosed and the species listed. The genus *Nemonchus* is synonymized with *Hoplolaimus* and *Gottholdsteineria* synonymized with *Rotylenchus*. *Spirotylenchus* is synonymized with *Rotylenchulus* and excluded from Hoplolaiminae. *Hoplolaimus coronatus* is synonymized with *Hoplolaimus galeatus*. *Scutellonema coheni* and *S. boocki* are synonymized with *S. brachyurum*. *Helicotylenchus nannus*, *Tylenchus spiralis* and *T. olaae* are synonymized with *Helicotylenchus dihystera* n. comb. *Helicotylenchus elisensis* is synonymized with *Rotylenchulus reniformis*. The following new combinations are proposed: *Scutellonema aberrans*; *Rotylenchus quartus*; *Rotylenchus pumilus*; *Helicotylenchus steueri* and *Rotylenchulus parvus*.

The following proposal for the classification of the described genera and species of the subfamily Hoplolaiminae Filipjev, 1934 is presented as the first paper on a revision of Hoplolaiminae. The purpose of this paper is to diagnose the subfamily and nominal genera, list their nominal species, and present a key to the genera and illustration of each genus.

Subsequent papers will give complete synonomy and references of the genera and species of Hoplolaiminae and propose new genera and species in this subfamily.

HISTORY

The subfamily Hoplolaiminae was proposed by Filipjev (1934) and diagnosed as follows: "The cuticle has peculiar rings, sometimes subdivided to give a scale-like appearance. There is a peculiar huge spear with proximal knobs". He did not designate a type genus, but included the following genera: *Paratylenchus* Micoletzky, 1922; *Atylenchus* Cobb, 1913; *Procriconema* Micoletzky, 1925; *Hoplolaimus* Daday, 1905; *Criconema* Hofmänner and Menzel, 1914; and *Iota* Cobb, 1913 (syn. *Ogma* Southern, 1914). As an illustration of this subfamily, he showed a picture of *Iota octangulare* Cobb, 1914 [= *Criconema octangulare* (Cobb, 1914) Taylor, 1936].

The subfamily Hoplolaiminae, as first proposed, was meant to include those genera that would now be primarily in the Criconematidae. Menzel (1917) had placed all the known species of *Criconema* Hofmänner and Menzel, 1914 in the

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[1]) Paper no. 1275, University of California Citrus Experiment Station, Riverside.

genus *Hoplolaimus*. Despite Cobb's 1923 paper redescribing the genus *Hoplolaimus* and showing it was not the same as *Criconema*, Filipjev (1934) proposed the subfamily Hoplolaiminae to include Criconematidae.

Filipjev (1936) transferred *Hoplolaimus* to the subfamily Tylenchinae, which also contained *Rotylenchus* Filipjev, 1936, pointing out the similarity of these two genera. Hoplolaiminae, therefore, became a synonym (in part) of Tylenchinae, but was not listed as such.

Chitwood & Chitwood's 1937 classification of the Nematoda listed the subfamily Hoplolaiminae, but genera were not included.

Steiner (1945) proposed the genus *Helicotylenchus* in the Tylenchinae to include those forms "having a modified terminal oesophagus with no distinctly set off bulb and with enlarged, protruding oesophageal glands". He concluded that Filipjev, when proposing the genus *Rotylenchus* to include these forms, "erred, however, in selecting *Tylenchus robustus* de Man, 1880 as the type species" as this species has a "distinct terminal bulb".

Thorne (1949) pointed out that de Man's *Tylenchus robustus* did have overlapping glands. Instead of synonymizing *Helicotylenchus* with *Rotylenchus*, he separated these genera on the basis of Steiner's drawing which showed amphid apertures at the base of the lip region.

Thorne's 1949 diagnosis of the subfamily Hoplolaiminae has been the basis for this subfamily to date. He included the genera *Hoplolaimus, Rotylenchus* and *Helicotylenchus*.

The classification of the Nematoda was revised by Chitwood & Chitwood (1950) and included the following genera in the Hoplolaiminae: *Hoplolaimus, Helicotylenchus, Rotylenchus, Tylenchorhynchus, Radopholus,* and *Pratylenchus*.

Goodey (1951) in his classification of the Hoplolaiminae did not recognize the genus *Helicotylenchus* and included *H. nannus* as a questionable synonym of *Rotylenchus erythrinae* (Zimmermann, 1904) Goodey, 1951. This classification of the Hoplolaiminae included the genera *Hoplolaimus* and *Rotylenchus*.

The genus *Rotylenchus* was revised by Golden (1956). He removed *Helicotylenchus* from synonymy and separated it from *Rotylenchus* on the distance from the dorsal esophageal gland orifice to the base of the spear. This work did not use the subfamily classification, but considered these genera as part of the family Tylenchidae.

The subfamily Hoplolaiminae was revised by Andrássy (1958 b). The basic classification of Golden (1956) was used for separating *Helicotylenchus* and *Rotylenchus*. Two more genera were proposed, *Scutellonema* and *Gottholdsteineria*, and the subfamily divided into two groups, the *Rotylenchus* group with *Helicotylenchus, Rotylenchus,* and *Gottholdsteineria,* and the *Hoplolaimus* group with *Scutellonema* and *Hoplolaimus*. A key to the genera and list of species in each genus was included in this paper.

Perry, Darling & Thorne (1959) revised the genus *Helicotylenchus*. They synonymized *Gottholdsteineria* with *Helicotylenchus* and proposed that all species of

spiral nematodes, except those with a large phasmid, be placed in the genus *Helicotylenchus*. No mention was made of the genus *Rotylenchus* or how it was to be separated from the genus *Helicotylenchus*.

Skarbilovich (1959) proposed the reclassification of the order Tylenchida Thorne, 1949 in which the subfamily Hoplolaiminae was diagnosed and included under the family Tylenchidae and the following genera listed: *Hoplolaimus, Rotylenchus,* and *Helicotylenchus*.

The family name Hoplolaimidae was used by Wieser (1953) in a discussion of marine nematodes for members of the Criconematidae. Chitwood (1958) proposed the family name Hoplolaimidae to include the subfamilies Heteroderinae Skarbilovich, 1947, Hoplolaiminae, Pratylenchinae Thorne, 1949, and Nacobbinae Chitwood, 1950. Heteroderidae Skarbilovich, 1947 was considered a synonym of Hoplolaimidae.

MATERIALS AND METHODS

Type specimens or type locality material has been obtained for most of the nominal species in this subfamily from nematologists throughout the world, and by collecting trips to type localities in the U.S. and Europe.

Over 14,000 mounted specimens of Hoplolaiminae have been assembled from many areas of the world. This material represents 70 nominal and new species and can be classified tentatively into the four recognized genera: *Hoplolaimus* 9 species, *Rotylenchus* 16 species, *Helicotylenchus* 32 species, and *Scutellonema* 13 species.

The specimens used in this study were usually fixed in F.A.A. (formalin, six parts; 95% alcohol, twenty parts; glacial acetic acid, one part; water, forty parts) or formalin (5%), then transferred to diluted glycerine, allowed to dehydrate slowly, and then mounted in dehydrated glycerine. The rapid glycerine techniques of Baker (1953) and Seinhorst (1959) and Franklin & Goodey's lactophenol method (1949) have also been used on some material. In addition, fresh specimens in water and in 5% formalin have been examined whenever possible.

Mayr, Linsley and Usinger (1953) has been used as a guide in systematics during the course of this study.

SYSTEMATICS

Subfamily Hoplolaiminae Filipjev, 1934

Hoplolaiminae Filipjev, Smithsonian Misc. Coll. 89, 1934: 33, in part; Chitwood & Chitwood, An Introduction to Nematology I, 1937: 49; Thorne, Proc. Helm. Soc. Wash. 16, 1949: 43; Chitwood & Chitwood, An Introduction to Nematology I (revised) 1950: 14; Goodey, Soil and Freshwater Nematodes, 1951: 107; Andrássy, Nematologica 3, 1958: 50; Skarbilovich, Acta Parasit. Polon. 7, 1959: 129.

Diagnosis emended. — Family Hoplolaimidae Wieser, 1953. Sexual dimorphism in the shape of the lip region. Stylet well developed, at least twice but no longer than four-and-a-half times body width at base of cephalic framework.

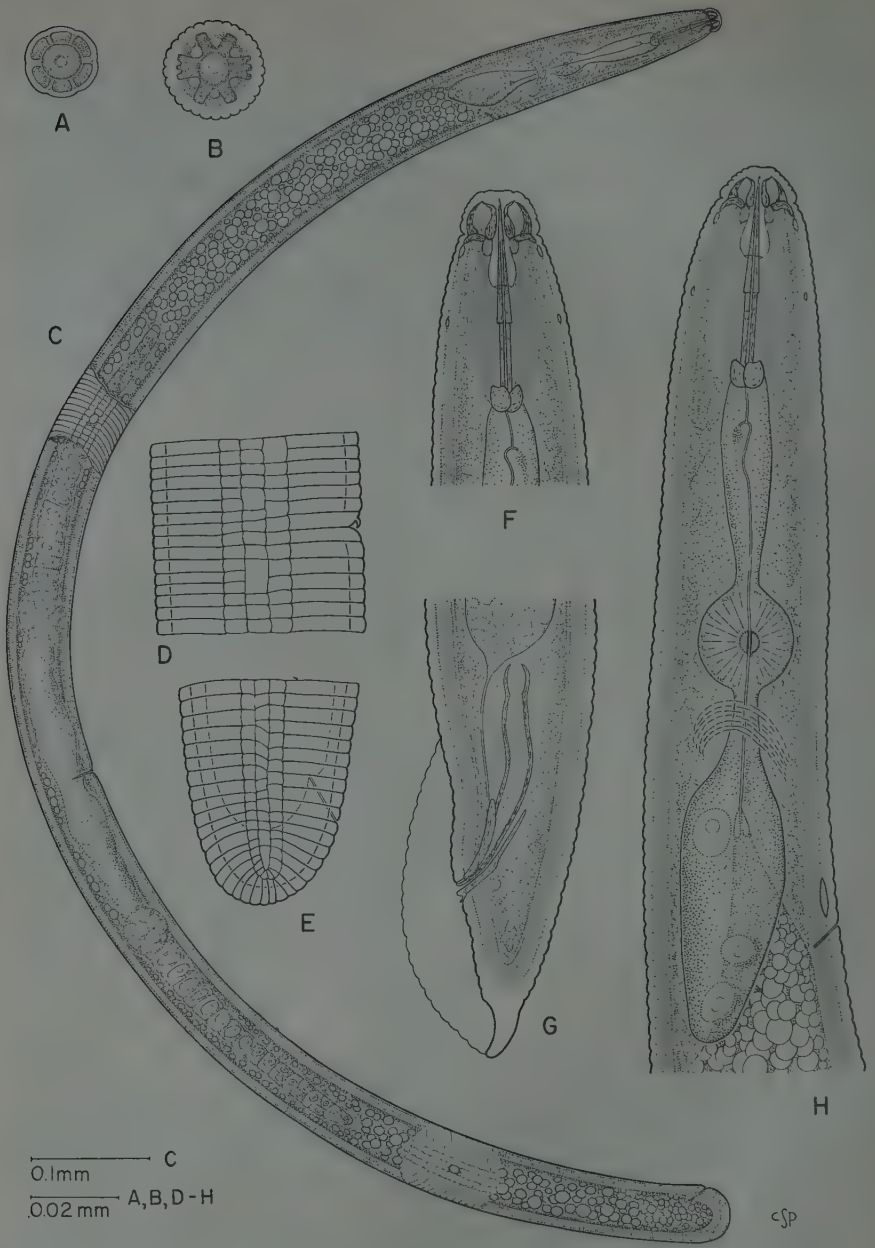

Fig. 1. *Hoplolaimus galeatus*. A. Female, face view. B. Female, cross-section through first annule of lip region. C. Female. D. Female, surface view at vulva. E. Female, tail. F. Male, anterior end. G. Male, tail. H. Female, anterior end.

Cephalic framework heavily cuticularized. Esophageal glands overlap intestine; esophageal intestinal cells at the juncture of esophagus and intestine. Ovaries outstretched, amphidelphic, didelphic; female tail short, not more than twice anal body diameter. Testis single, outstretched; caudal alae enveloping male tail. Four or fewer incisures (involutions) in the lateral field.

Type genus: *Hoplolaimus* Daday, 1905 by subsequent designation (Thorne, 1949)

syn. *Nemonchus* Cobb, 1913

Nominal genera included:

Rotylenchus Filipjev, 1936
syn. *Gottholdsteineria* Andrássy, 1958 new synonomy
Helicotylenchus Steiner, 1945
Scutellonema Andrássy, 1958

Specimens obtained from the type locality of the genus *Nemonchus* and identified as such, show that the genera *Nemonchus* and *Hoplolaimus* are the same.

Perry *et al* (1959) proposed *Gottholdsteineria* Andrássy, 1958 as a synonym of *Helicotylenchus*. Examination of specimens that Andrássy included in the genus *Gottholdsteineria* shows that they are similar to specimens in the genus *Rotylenchus* Filipjev, 1936. *Gottholdsteineria* is therefore proposed as a synonym of *Rotylenchus*.

Spirotylenchus Lordello and Cesnik, 1958 was described as a new genus of Hoplolaiminae. The illustrations and description of this genus and specimens from near the type locality indicate that these are immature females and males of the genus *Rotylenchulus* Linford and Oliveira, 1940. *Spirotylenchus* is therefore proposed as a synonym of *Rotylenchulus*.

Key to the genera of Hoplolaiminae

1. Esophageal glands overlap intestine ventrally, laterally and dorsally; typically, greatest length ventrally . . . . . *Helicotylenchus*
   Esophageal glands overlap intestine dorsally and laterally; greatest length dorsally . . 2
2. Phasmids small . . . . . *Rotylenchus*
   Phasmids enlarged (scutella) 3
3. Phasmids located posterior to vulva . . . . . *Scutellonema*
   One phasmid above vulva, one below vulva . . . . . *Hoplolaimus*

Genus *Hoplolaimus* Daday, 1905

Diagnosis emended. Hoplolaiminae. Lip region with longitudinal striations, set off from body. Spear knobs with anterior projections. Dorsal esophageal gland opening near base of spear knobs (¼ or less the spear length). Esophageal glands overlap intestine dorsally and laterally. Female tail round, shorter than width of body at anus. Phasmids (scutella) enlarged, one anterior to vulva and one posterior to vulva. Four or fewer incisures in the lateral field.

Type species *Hoplolaimus tylenchiformis* Daday, 1905

Nominal species included:

H. galeatus (Cobb, 1913) Thorne, 1935
syn. *Nemonchus galeatus* Cobb, 1913
Hoplolaimus coronatus Cobb, 1923
H. proporicus Goodey, 1957
H. seinhorsti Luc, 1958
H. angustalatus Whitehead, 1959

The type specimen of *Hoplolaimus tylenchiformis* Daday, 1905 was redescribed by Andrássy in 1954. In 1958 Andrássy proposed *Hoplolaimus coronatus* (= *H. galeatus*) as a synonym of *H. tylenchiformis*. *Hoplolaimus galeatus* collected from the type locality (Fig. 1) exhibits many differences from Andrássy's redescription of 1954, especially in the lip region annulation and number of lateral striations on the first lip region annule. The type specimen of *H. tylenchiformis* was lost in 1956, but conversations with Dr. Andrássy in 1959 confirmed that the lip region is as he described it in 1954. Consequently *H. galeatus* is reinstated as a valid species.

Thorne (1935) pointed out the probable identity of *Nemonchus galeatus* Cobb, 1913 with *Hoplolaimus coronatus* Cobb, 1923. Filipjev & Schuurmans Stekhoven (1941) listed *Nemonchus galeatus* as a synonym of *Hoplolaimus coronatus*. Specimens in Cobb's collection at Beltsville labeled Arlington Farms, the type locality of *Nemonchus galeatus*, and specimens collected at Arlington Farms are the same as type locality specimens of *Hoplolaimus coronatus*. Therefore, because of the law of priority, *Hoplolaimus coronatus* is proposed as a synonym of *Hoplolaimus galeatus* (Cobb, 1913) Thorne, 1935.

Genus *Scutellonema* Andrássy, 1958

Diagnosis emended. Hoplolaiminae. Lip region with or without longitudinal striations. Spear knobs rounded. Dorsal esophageal gland opening near base of spear knobs (¼ or less spear length). Esophageal gland overlaps intestine dorsally and laterally. Female tail round. Phasmids enlarged, located posterior to vulva. Four or fewer incisures in the lateral field.

Type species *Scutellonema blaberum* (Steiner, 1937) Andrássy, 1958

syn. *Rotylenchus blaberus* Steiner, 1937

Nominal species included:

S. bradys (Steiner & LeHew, 1933) Andrássy, 1958
syn. *Hoplolaimus bradys* Steiner & LeHew, 1933
Anguillulina bradys (Steiner & LeHew, 1933) Goodey, 1935
Rotylenchus bradys (Steiner & LeHew, 1933) Filipjev, 1936
S. brachyurum (Steiner, 1938) Andrássy, 1958
syn. *Rotylenchus brachyurus* Steiner, 1938
Rotylenchus coheni Goodey, 1952
Scutellonema coheni (Goodey, 1952) Andrássy, 1958
Rotylenchus boocki Lordello, 1957
Scutellonema boocki (Lordello, 1957) Andrássy, 1958

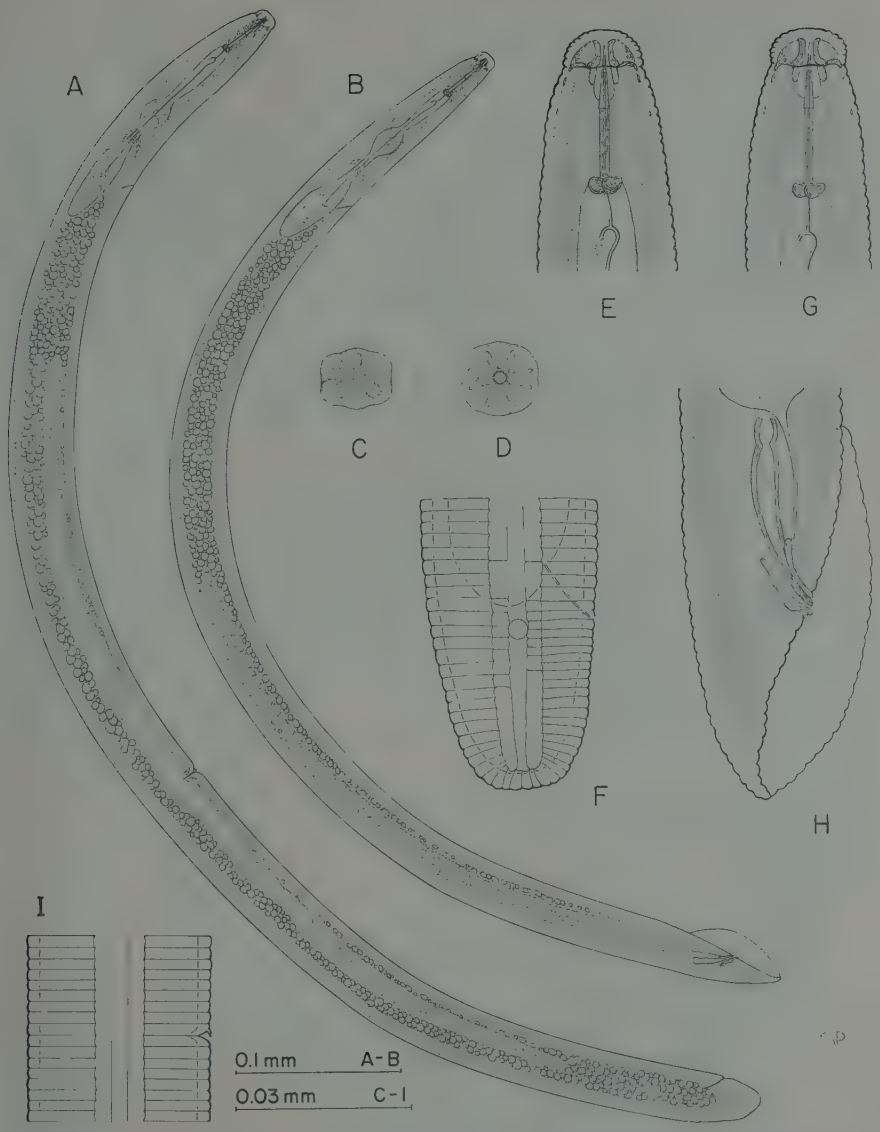

Fig. 2. *Scutellonema bradys*. A. Female. B. Male. C. Female, face view. D. Female, cross-section through first annule of lip region. E. Female, anterior end. F. Female, surface view posterior end. G. Male, anterior end. H. Male, posterior end. I. Female, surface view at vulva.

S. christiei (Golden & Taylor, 1956) Andrássy, 1958
syn. *Rotylenchus christiei* Golden & Taylor, 1956
S. clathricaudatum Whitehead, 1959
S. dioscoreae Lordello, 1959
S. aberrans (Whitehead, 1959) n. comb.
syn. *Hoplolaimus aberrans* Whitehead, 1959

Type material of *S. coheni* has been compared with specimens from the type locality of *S. brachyurum*. No morphological differences have been noted. Type locality material of *S. boocki* has been compared with specimens from the type locality of *S. brachyurum*. No morphological differences have been seen between these specimens or the description and illustrations of *S. boocki* and specimens of *S. brachyurum*.

The type specimen of *Hoplolaimus aberrans* has been examined and determined to be a *Scutellonema*.

Specimens from the type host (*Dioscorea* sp.) and type locality (Jamaica) were used to draw the illustrations of *Scutellonema bradys* (Fig. 2).

Genus *Rotylenchus* Filipjev, 1936

syn. *Gottholdsteineria* Andrássy, 1958

Diagnosis emended. Hoplolaiminae. Lip region with longitudinal striations. Dorsal esophageal opening near base of spear knobs (¼ or less the spear length). Esophageal glands overlap intestine dorsally, laterally, and slightly ventrally. Phasmids small, near anus. Female tail round or slightly convex dorsally. Four incisures in the lateral field.

Type species *Rotylenchus robustus* (de Man, 1876) Filipjev, 1936

syn. *Tylenchus robustus* de Man, 1876
Tylenchorhynchus robustus (de Man, 1876) Micoletzky, 1922 (in part)
Anguillulina robusta (de Man, 1876) Goodey, 1932

Nominal species included:

R. uniformis (Thorne, 1949) Loof & Oostenbrink, 1958
syn. *Hoplolaimus uniformis* Thorne, 1949
R. buxophilus Golden, 1956
syn. *Gottholdsteineria buxophila* (Golden, 1956) Andrássy, 1958
Helicotylenchus buxophilus (Golden, 1956) Perry, 1959
R. gracilidens (Sauer, 1958) Sauer, 1958
syn. *Hoplolaimus gracilidens* Sauer, 1958
R. quartus (Andrássy, 1958) n. comb.
syn. *Gottholdsteineria quarta* Andrássy, 1958
Helicotylenchus quarta (Andrássy, 1958) Perry, 1959
R. pumilus (Perry, 1959) n. comb.
syn. *Helicotylenchus pumilus* Perry in Perry, Darling & Thorne, 1959
R. goodeyi Loof & Oostenbrink, 1958
syn. *Gottholdsteineria goodeyi* (Loof & Oostenbrink, 1958) Andrássy, 1958

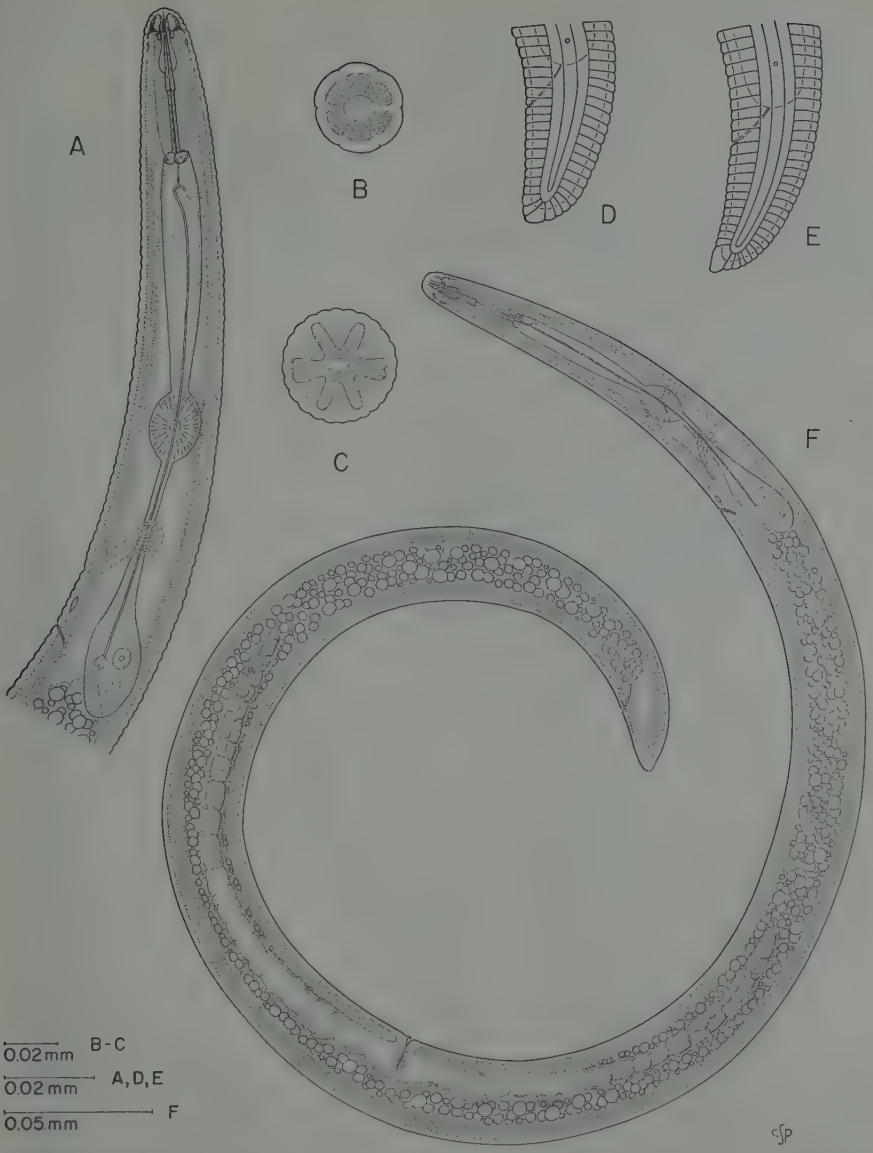

Fig. 3. *Rotylenchus buxophilus*. A. Female, anterior end. B. Female, face view. C. Female, cross-section through first annule of lip region. D-E. Female, surface view of posterior end. F. Female.

Species inquirendae:

Rotylenchus pararobustus (Schuurmans Stekhoven & Teunissen, 1938) Filipjev & Schuurmans Stekhoven, 1941
syn. *Tylenchorhynchus pararobustus* Schuurmans Stekhoven & Teunissen, 1938
Gottholdsteineria pararobusta (Schuurmans Stekhoven & Teunissen, 1938) Andrássy, 1958

Genus *Helicotylenchus* Steiner, 1945

Diagnosis emended. Hoplolaiminae. Lip region without longitudinal striations, not set off from body. Dorsal esophageal gland opening behind spear knobs variable (usually ¼ or more the spear length). Esophageal glands overlap esophagus ventrally, laterally and dorsally. Female tail round to convex-concave dorsally. Phasmids small, near anus. Four incisures in the lateral field.

Type species *Helicotylenchus dihystera* (Cobb, 1893) n. comb.

syn. *Tylenchus dihystera* Cobb, 1893
Tylenchus olaae Cobb, 1906
Aphelenchus dubius var. *peruensis* Steiner, 1920
Tylenchus spiralis Cassidy, 1930
Helicotylenchus nannus Steiner, 1945

Nominal species included:

H. multicinctus (Cobb, 1893) Golden, 1956
syn. *Tylenchus multicinctus* Cobb, 1893
Tylenchorhynchus multicinctus (Cobb, 1893) Micoletzky, 1922
Anguillulina multicincta (Cobb, 1893) Goodey, 1932
Rotylenchus multicinctus (Cobb, 1893) Filipjev, 1936
H. erythrinae (Zimmermann, 1904) Golden, 1956
syn. *Tylenchus erythrinae* Zimmermann, 1904
Anguillulina erythrinae (Zimmermann, 1904) Goodey, 1940
Rotylenchus erythrinae (Zimmermann, 1904) Goodey, 1951
H. pseudorobustus (Steiner, 1914) Golden, 1956
syn. *Tylenchus pseudorobustus* Steiner, 1914
H. africanus (Micoletzky, 1916) Andrássy, 1958
syn. *Tylenchus africanus* Micoletzky, 1916
Tylenchorhynchus africanus (Micoletzky, 1916) Micoletzky, 1922
Rotylenchus africanus (Micoletzky, 1916) Filipjev, 1936
H. steueri (Stefánski, 1916) n. comb.
syn. *Aphelenchus steueri* Stefánski, 1916
Tylenchorhynchus steueri (Stefánski, 1916) Micoletzky, 1922
H. iperoiguensis (Carvalho, 1956) Andrássy, 1958
syn. *Rotylenchus iperoiguensis* Carvalho, 1956
H. melancholicus (Lordello, 1955) Andrássy, 1958
syn. *Rotylenchus melancholicus* Lordello, 1955
H. microlobus Perry in Perry, Darling and Thorne, 1959
H. platyurus Perry in Perry, Darling and Thorne, 1959
H. digonicus Perry in Perry, Darling and Thorne, 1959.

Type locality material of *Tylenchus dihystera* Cobb, 1893, *Tylenchus olaae* Cobb, 1906, *Tylenchus spiralis* Cassidy, 1930, and specimens from near the type locality (Tingo Mario, Huaras and Yungay, Peru) of *Aphelenchus dubius* var. *peruensis* Steiner, 1920 cannot be distinguished from syntypes of *Helicotylenchus nannus*. It is therefore proposed, under the rules of priority, that *Tylenchus olaae, Tylenchus spiralis, Aphelenchus dubius* var. *peruensis* and *Helicotylenchus nannus* become synonyms of *Helicotylenchus dihystera* (Cobb, 1893) n. comb. (Fig. 4).

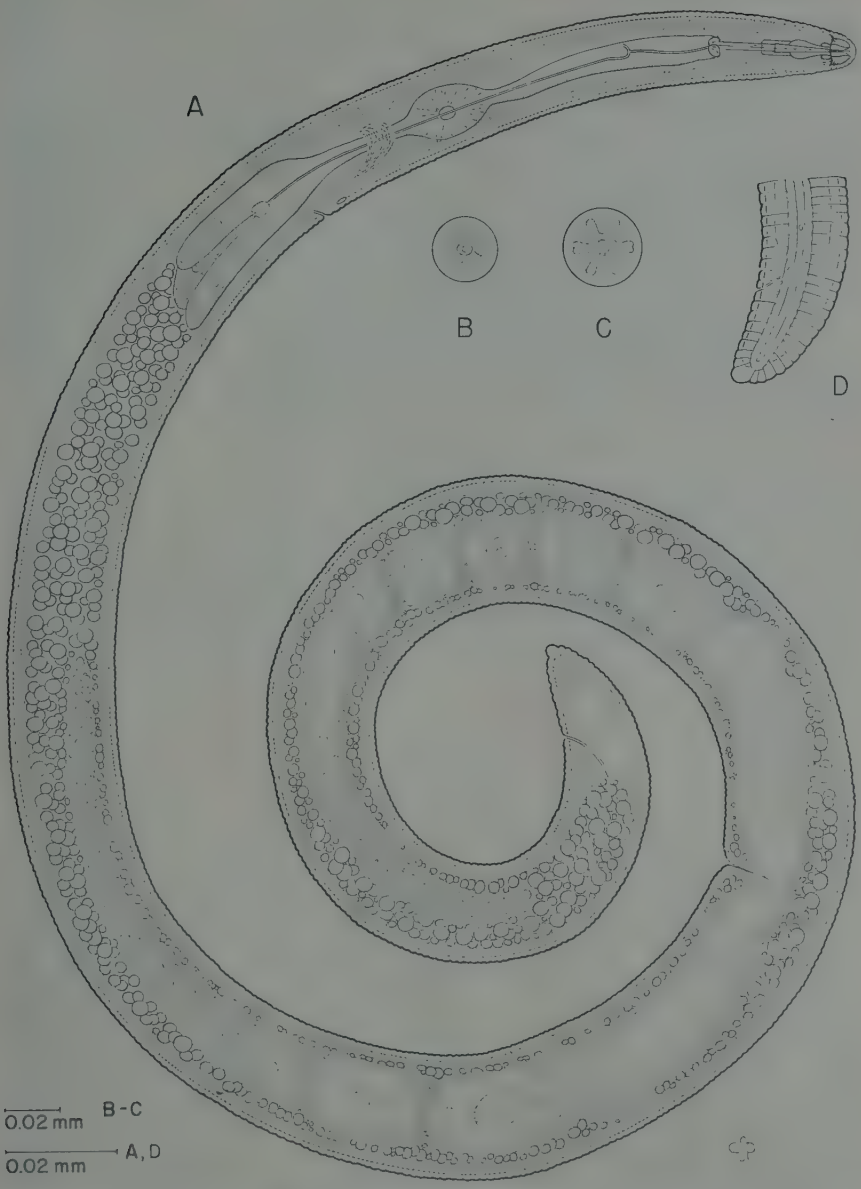

Fig. 4. *Helicotylenchus dihystera.* A. Female. B. Female, face view. C. Female, cross-section through first annule of lip region. D. Female, surface view of posterior end.

Specimens collected from the type locality of *Tylenchus pseudorobustus* Steiner, 1914 and near the type locality (edge of the Inn River, Austria) of *Aphelenchus steueri* Stefánski, 1916 show that these are valid species of the genus *Helicotylenchus*.

Specimens from near the type locality of *Tylenchus africanus* Micoletzky, 1916 (edge of Victoria Falls, Zambesi River) are similar to Micoletzky's description and figures of this species. *Helicotylenchus africanus* (Micoletzky, 1916) Andrássy, 1958 is therefore considered a valid species.

Helicotylenchus elisensis (Carvalho, 1957) Carvalho, 1959 (synonym *Rotylenchus elisensis* Carvalho, 1957) from the type locality indicates that it is the same as immature females and males of *Rotylenchulus reniformis* Linford and Oliveira, 1940. *H. elisensis* is therefore proposed as a synonym of *R. reniformis*.

The description and illustrations of *Helicotylenchus parvus* Williams, 1960 indicate that it is the immature female of the genus *Rotylenchulus*. It is therefore proposed as *Rotylenchulus parvus* (Williams, 1960) n. comb.

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RÉSUMÉ

Revision des Hoplolaiminae (Nematoda)

I. Classification des genres et espèces nominaux

L'auteur donne une revision de tous les genres et espèces proposés pour la sous-famille des Hoplolaiminae et propose une classification des genres et espèces nominaux. Il donne la diagnose de la sous-famille et des genres nominaux et la liste des espèces nominales pour les genres. Le genre *Nemonchus* Cobb, 1933 est considéré comme synonyme de *Hoplolaimus* Daday, 1905. *Spirotylenchus* Lordello & Cesnik, 1958 est proposé comme un synonyme de *Rotylenchulus* Linford & Oliveira, 1940 et est exclu des *Hoplolaiminae*. *Gottholdsteineria* Andrássy, 1958 est proposé comme un synonyme de *Rotylenchus* Filipjev, 1936.

Hoplolaimus coronatus Cobb, 1923 est proposé comme un synonyme de *Hoplolaimus galeatus* (Cobb, 1913) Thorne, 1935. *Scutellonema coheni* (Goodey, 1952) Andrássy, 1958 et *Scutellonema boocki* (Lordello, 1957) Andrássy, 1958 sont proposés comme synonymes de *Scutellonema brachyurum* (Steiner, 1938) Andrássy 1958. *Helicotylenchus nannus* Steiner, 1945. *Tylenchus spiralis* Cassidy, 1930 et *Tylenchus oleae* Cobb, 1906 sont proposés comme synonymes de *Helicotylenchus dihystera* (Cobb, 1893) n. comb. *Helicotylenchus elisensis* (Carvalho, 1957) Carvalho, 1959 est proposé comme un synonyme de *Rotylenchulus reniformis*, Linford & Oliveira, 1940. Les nouvelles combinaisons suivantes sont proposées: *Scutellonema aberrans* (Whitehead, 1959) n. comb.; *Rotylenchus quartus* (Andrássy, 1958) n. comb.; *Rotylenchus pumilus* (Perry, 1959) n. comb.; *Helicotylenchus steueri* (Stefánski, 1916) n. comb. et *Rotylenchus parvus* (Williams, 1960) n. comb.

РЕЗЮМЕ

Произведен обзор и предложена классификация родов и видов нематод подсемейства Hoplolaiminae (70 видов: Hoplolaimus-9, *Rotylenchus*-16, *Helicotylenchus*-32, *Scutellonema*-13). Даны диагнозы подсемейства и родов, с определительной таблицей последних; перечислены виды каждого рода.

Род *Nemonchus* Cobb, 1913 является синонимом *Hoplolaimus* Daday, 1905. *Spirotylenchus* Lordello and Cesnik, 1958 предлагается как синоним *Rotylenchulus* Linford and Oliveira, 1940 и исключается из Hoplolaiminae. *Gottholdsteineria* Andrássy, 1958 предлагается как синоним *Rotylenchus* Filipjev, 1936.

Hoplolaimus coronatus Cobb, 1923 предлагается как синоним *Hoplolaimus galeatus* (Cobb, 1913) Thorne, 1935. *Scutellonema coheni* (Goodey, 1952) Andrássy, 1958 и *Scutellonema boocki* (Lordello, 1957) Andrássy, 1958 предлагаются как синонимы *Scutellonema brachyurum* (Steiner, 1938) Andrássy, 1958. *Helicotylenchus nannus* Steiner, 1945, *Tylenchus spiralis* Cassidy, 1930 и *Tylenchus olaae* Cobb, 1906 предлагаются как синонимы *Helicotylenchus dihystera* (Cobb, 1893) n. comb. *Helicotylenchus elisensis* (Carvalho, 1957) Carvalho 1959 предлагается как синоним *Rotylenchulus reniformis* Linford and Oliveira, 1940.

Предлагаются следующие новые комбинации наименований: *Scutellonema aberrans* (Whitehead, 1959) n. comb.; *Rotylenchus quartus* (Andrássy, 1958) n. comb.; *Rotylenchus pumilus* (Perry, 1959) n. comb.; *Helicotylenchus steueri* (Stefanski, 1916) n. comb. и *Rotylenchulus parvus* (Williams, 1960) n. comb.

REFERENCES

Andrássy, I. (1954). Über einige van Daday beschriebene Nematoden-Arten. *Zool. Anz.* **152**, 138-144.

—— (1958a). Erd- und Süsswassernematoden aus Bulgarien. *Acta Zool.* **4**, 1-88.

—— (1958b). *Hoplolaimus tylenchiformis* Daday, 1905 (Syn. *H. coronatus* Cobb, 1923) und die Gattungen der Unterfamilie Hoplolaiminae Filipjev, 1936. *Nematologica* **3**, 44-56.

Baker, A. D. (1953). Rapid method for mounting nematodes in glycerine. *Canad. Entom.*, **85**, 77-78.

Carvalho, J. C. (1956). *Helicotylenchus nannus* (description of the male) and *Rotylenchus iperoiguensis* n. sp. *Rev. Inst. Lutz* **16**, 142-147.

—— (1957). *Rotylenchus elisensis* nova espécia associada com raizes de soja. *Rev. Inst. Adolfo Lutz* **17**, 43-46.

—— (1959). *Helicotylenchus elisensis* n. comb. (Nematoda: Tylenchidae). *Arch. Instit. Biol. S. Paulo* **26**, 45-48.

Cassidy, Gertrude. (1930). Nematodes associated with sugar cane in Hawaii. *Hawaii. Plant. Rec.* **34**, 379-387.

Chitwood, B. G. (1958). The classification of plant-parasitic nemas and related forms. *XVth Int. Congr. Zool., Sect. VIII, paper* **28**, 1-2.

Chitwood, B. G., & Chitwood, M. B. (1937). *An introduction to nematology. Section* I, *Part* I. Baltimore, Md., Monumental Printing Co., 53 pp.

—— & —— (1950). *An introduction to nematology.* (Revised) *Section* I. Baltimore, Md., Monumental Printing Co., 213 pp.

Cobb, N. A. (1893). Nematode worms found attacking sugar-cane. (In Plant diseases and their remedies). *Agr. Gaz. N. S. Wales* **4**, 803-833.

—— (1906). Free living nematodes inhabiting the soil about the roots of cane, and their relation to root diseases. (In Fungus maladies of the sugar cane, with notes on associated insects and nematodes). *Bull. Hawaii. Sug. Ass. Path. Physiol.* **5**, 163-195.

—— (1913). New nematode genera found inhabiting fresh water and non-brackish soils. *J. Wash. Acad. Sci.* **3**, 432-444.

—— (1923). An amendation of *Hoplolaimus* Daday, 1905 nec auctores. *J. Wash. Acad. Sci.* **13**, 211-214.

Daday, J. (1905). Untersuchungen über die Süsswasser-Mikrofauna Paraguays. *Zoologica, Stuttgart*, **18**, 1-349.

Filipjev, I. N. (1934). The classification of the free-living nematodes and their relation to the parasitic nematodes. *Smithson. misc. Coll.* **89**, 1-63.

—— (1936). On the classification of the Tylenchinae. *Proc. helm. Soc. Wash.* **3**, 80-82.

Filipjev, I. N., & Schuurmans Stekhoven, Jr, J. H. (1941). *A manual of agricultural helminthology.* Leiden, Brill, 878 pp.

Franklin, Mary T., & Goodey, J. B. (1949). A cotton blue-lactophenol technique for mounting plant-parasitic nematodes. *J. Helm.* **23**, 175-178.

Golden, A. M. (1956). Taxonomy of the spiral nematodes (*Rotylenchus* and *Helicotylenchus*), and the developmental stages and host-parasite relationships of *R. buxophilus,* n. sp. attacking boxwood. *Bull. Md. agric. Expt. Sta.* **A-85**, 1-28.

Golden, A. M., & Taylor, A. L. (1956). *Rotylenchus christiei,* n. sp. a new spiral nematode species associated with roots of turf. *Proc. helm. Soc. Wash.* **23**, 109-112.

Goodey, J. B. (1952). *Rotylenchus coheni* n. sp. (Nematoda: Tylenchida) parasitic on the roots of *Hippeastrum* sp. *J. Helm.* **26**, 91-96.

——(1957). *Hoplolaimus proporicus* n. sp. (Hoplolaiminae: Tylenchida). *Nematologica* **2**, 108-113.

Goodey, J. B., & Seinhorst, J. W. (1960). Further observations and comments on the identity of *Rotylenchus robustus* (de Man, 1876) Filipjev, 1934 with a description of a proposed neotype and a new definition of *Rotylenchus goodeyi. Nematologica* **5**, 136-148.

Goodey, T. (1932). The genus *Anguillulina* Gerv. and v. Ben., 1859, vel *Tylenchus* Bastian, 1865. *J. Helm.* **10**, 75-180.

—— (1935). Observations on a nematode disease of yams. *J. Helm.* **13**, 173-190.

—— (1951). *Soil and freshwater nematodes.* London, Methuen, 390 pp.

Linford. M. B., & Oliveira, Juliette, M. (1940). *Rotylenchus reniformis,* nov. gen., n. sp., a nematode parasite of roots. *Proc. helm. Soc. Wash.* **7**, 35-42.

Loof, P. A. A., & Oostenbrink, M. (1958). Die Identität von *Tylenchus robustus* de Man. *Nematologica* **3**, 34-43.

Lordello, L. G. E. (1955). A new nematode, *Rotylenchus melancholicus,* n. sp. found associated with grass roots, and its sexual dimorphism. *J. Wash. Acad. Sci.* **45**, 81-83.

—— (1957). A note on nematode parasites of red anthurium (*Anthurium andraeanum* Lind.) with a description of *Rotylenchus boocki* n. sp. *Nematologica* **2**, 273-276.

—— (1959). A nematosis of yam in Pernambuco, Brazil, caused by a new species of the genus "Scutellonema." *Rev. Brasil Biol.* **19**, 35-41.

Lordello, L. G. E., & Cesnik, R. (1958). Alguns nematódeos do tomateiro. *Rev. Brasil. Biol.* **18**, 159-165.

Luc, M. (1958). Les nematodes et le flétrissement des cotonniers dans le Sud-ouest de Madagascar. *Cot. et Fibr. trop.* **13**, 1-18.

De Man, J. G. (1876). Onderzoekingen over vrij in de aarde levende Nematoden. *Tijdschr. Ned. dierk. Ver.* **2**, 78-196.

Mayr, E., Linsley, E. G., & Usinger, R. L. (1953). *Methods and principles of systematic zoology.* New York, McGraw-Hill, 32 pp.

Menzel, R. (1917). Zur Kenntnis der freilebenden Nematodengattung *Hoplolaimus* v. Daday, *Rev. Suisse Zool.* **25**, 153-162.

Micoletzky, H. (1916). Süsswasser-Nematoden aus Südafrika. *Denkschr. K. Akad. Wiss. Wien* **92**, 149-171.

—— (1922). Die freilebenden Erd-Nematoden mit besonderer Berücksichtigung der Steiermark, und der Bukowina, zugleich mit einer Revision sämtlicher nicht mariner, freilebender Nematoden in Form von Genus-Beschreibungen und Bestimmungsschlüsseln. *Arch. Naturgesch.* 87, A, 1-650.

Perry, V. G., Darling, H. M., & Thorne, G. (1959). Anatomy, taxonomy, and control of certain spiral nematodes attacking blue grass in Wisconsin. *Bull. Wis. agric. Expt. Sta.* **207**, 1-24.

Sauer, M. R. (1958). *Hoplolaimus gracilidens, Radopholus inaequalis,* and *Radopholus neosimilis,* three new tylenchs native to Victoria, Australia. *Nematologica* **3**, 97-107.

Seinhorst, J. W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica* **4**, 67-69.

Schuurmans Stekhoven, Jr. J. H., & Teunissen, R. J. H. (1938). *Nématodes libres terrestres,* Explor. Parc. Nat. Albert, Mission de Witte (1933-35), **22**, Bruxelles, Inst. parcs nat. Congo Belge, 1-229.

Skarbilovich, T. S. (1959). On the structure of systematics of nematodes order Tylenchida Thorne, 1949. *Acta parasit. Polon.* **7**, 117-132.

Stefanski, W. (1916). Die freilebenden Nematoden des Inn, ihre Verbreitung und Systematik. *Zool. Anz.* **46**, 369-385.

STEINER, G. (1914). Freilebende Nematoden aus der Schweiz. I. Teil einer vorläufigen Mitteilung. *Arch. Hydrobiol.* (*Plankt.*) **9**, 259-276.

—— (1920). Freilebende Süsswassernematoden aus peruanischen Hochgebirgsseen. *Rev. Suisse Zool.* **28**, 11-44.

—— (1937). Opuscula miscellanea, V. (2) *Rotylenchus blaberus* n. sp., a nematode parasite of yams (*Dioscorea* sp.). *Proc. helm. Soc. Wash.* **4**, 34-35.

—— (1938). Nematodes infesting red spiderlilies. *J. agric. Res.* **56**, 1-8.

—— (1945). *Helicotylenchus*, a new genus of plant-parasitic nematodes and its relationship to *Rotylenchus* Filipjev. *Proc. helm. Soc. Wash.* **12**, 34-38.

STEINER, G., & LE HEW, ROWENA R. (1933). *Hoplolaimus bradys*, n. sp. (Tylenchidae, Nematodes), the cause of a disease of yam (*Dioscorea* sp.). *Zool. Anz.* **101**, 260-264.

THORNE, G. (1935). Notes on free-living and plant-parasitic nematodes II. *Proc. helm. Soc. Wash.* **2**, 96-98.

—— (1949). On the classification of the *Tylenchida*, new order (Nematoda, Phasmida). *Proc. helm. Soc. Wash.* **16**, 37-73.

WHITEHEAD, A. G. (1959). *Hoplolaimus angustalatus* n. sp. (Hoplolaiminae: Tylenchida). *Nematologica* **4**, 99-105.

—— (1959). *Hoplolaimus aberrans* n. sp. (Hoplolaiminae: Tylenchida). *Nematologica* **4**, 268-271.

WIESER, W. (1953). Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. *Arch. Zool.* **4**, 439-484.

WILLIAMS, J. R. (1960). Studies on the nematode soil fauna of sugar cane fields in Mauritius. 4. Tylenchoidea (partim). *Occas. Pap. Mauritius Sugar Ind. Res. Inst.* 4, 1-30.

ZIMMERMANN, A. (1904). Eenige pathologische en physiologische waarnemingen over koffie. *Meded. PlTuin, Batavia* **67**, 1-105.

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LONGEVITY OF *RADOPHOLUS SIMILIS* (COBB) IN HOST-FREE SOIL [1])

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Burrowing nematodes do not survive more than six months in sandy soils in Central Florida (U.S.A.) in the absence of suscept roots. Populations in citrus roots and soil contained in cheesecloth, nylon, or polyethylene bags buried at depths of 31-152 cm disappeared within six months, whereas *Hoplolaimus coronatus, Pratylenchus brachyurus,* and numerous saprobic species survived under these conditions. Polyethylene was not toxic to burrowing nematodes, and was impenetrable to this species in greenhouse tests. Living uninfected or infected citrus roots in the vicinity of buried polyethylene bags of infested roots and soil had no effect in prolonging the longevity of the *Radopholus* contained in those bags. Burrowing nematodes in soil and roots enclosed in polyethylene failed to survive when stored six months at an average laboratory temperature of 24.4° C. and an average refrigerator temperature of 1.1° C. Additional bags of infested material placed in gallon cans of autoclaved soil and held six months at an average greenhouse temperature of 24.9° C. likewise failed to yield burrowing nematodes when the cans were either kept dry, maintained at field capacity, or placed on an alternating cycle from field capacity to dry conditions.

The control program in Florida (U.S.A.) has sought to prevent or reduce the spread of the burrowing nematode *Radopholus similis,* the causal agent of spreading decline of citrus, by total eradication of the pathogen within sites previously occupied by infected citrus groves. Having delimited the extent of infection, all citrus trees within the area, as well as a margin two trees wide around it, are removed and the soil is fumigated with dichloropropene-dichloropropane (D-D) at the rate of 227 l per 0.4 ha (60 gal. per acre) (Suit & DuCharme, 1957). An attempt is then made to maintain the treated area completely free of vegetation for six months, after which a non-citrus crop resistant to the burrowing nematode may be planted. Two years after fumigation, the treated area may again be planted to citrus, provided the burrowing nematode is not present, as determined by examination. Success of the program, among other factors, has hinged upon the maximum period of time that *R. similis* can exist without food.

Little work has been done on the longevity of *R. similis*. Van der Vecht (1950) believed that females of this species could exist in moist soil for a considerable time, although larvae had a shorter life in such an environment. DuCharme (1956) found that burrowing nematodes survived eight weeks without food when kept under laboratory conditions in a column of moist soil 102 cm long. A related species, *R. oryzae* (Van Breda de Haan), survived for more than 10 weeks in soil with or without severed rice plant roots (Van der Vecht & Bergman, 1952). These

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investigators concluded "that severed roots in a moist soil will harbour living *R. oryzae* for a considerable time." The first detailed tests on the longevity of the burrowing nematode infecting citrus were made by Birchfield (1957) who tested survival of the species on agar at room temperature, in 4.7-l containers of soil placed in a temperature tank maintained at 23.3-24.4° C and in 3.8 l or 18.9 l containers buried in the soil at a depth of 91 cm. He concluded that the burrowing nematode was unable to survive in soil for more than four months without food.

The work herein was started in 1956 to test the assumption that burrowing nematodes could not survive for more than two years in the absence of food under diverse conditions.

METHODS AND RESULTS

Survival of Burrowing Nematodes in Field Soil

In the first of four tests, half-pint quantities of chopped, burrowing nematode-infected citrus roots and infested subsoil were placed in cheesecloth bags of double thickness and buried 61 cm apart at each of five depths from 31 to 152 cm in an area formerly occupied by nematode-infected citrus trees. This grove had been eradicated and the soil fumigated twice with D-D, 14 months apart. The present test was started six months after the last fumigation. Weeds appearing on the test site during the experiment were eliminated manually. At monthly intervals, one bag from each depth was examined for burrowing nematodes using a modified Oostenbrink apparatus (Malo, 1960 and Tarjan, Simanton & Russell, 1956).

The second test was made along the same lines as the first except for these differences: 1) Twelve 250 ml samples of chopped, burrowing nematode-infected roots and infested soil were enclosed in parachute nylon cloth or in one mil-thick polyethylene bags and buried at depths of 61, 91, 122, and 152 cm. 2) The herbicide Karmex DW (80 percent 3-3, 4-dichlorophenyl-1, 1-dimethyl urea) was applied at the rate of 9.1 kg per 0.4 ha as a deterrent to weed growth. 3) All retrieved samples were processed in the Seinhorst elutriator (Seinhorst, 1956), and the wash water from each sample was saved. The following morning the supernatant water was decanted, and the settled debris was then placed around the roots of an okra plant used as an indicator host.

Another test using polyethylene bags was made in a burrowing nematode-infested orange grove. Here an attempt was made to discover whether burial of plastic bags containing burrowing nematodes in the vicinity of living nematode-infected citrus roots might predispose the nematodes toward longer life despite enclosure in a food-free environment. Six 473 cc samples of chopped, infected roots and infested soil containing an average of 118 burrowing nematodes each were placed in plastic bags and buried at a depth of 91 cm, 31 cm apart from one another. One sample was retrieved at monthly intervals for a period of six months and examined for nematodes.

Finally, 15 polyethylene bags containing 237 cc of chopped, infected roots mixed with infested soil were buried at a depth of 91 cm in the infested grove

used for the test immediately preceding. At intervals of six, eight, and 10 months after burial, five samples were examined for burrowing nematodes.

Results from these tests appear in Table I. In Test 1, after six months, the cheesecloth surrounding the samples remaining in the soil had deteriorated, precluding further recovery of samples. In Test 2, the use of okra as an indicator plant for *R. similis* that may have passed through the retaining sieves with wash water proved fruitless; positive infections were detected only where the species had already been observed by direct examination of the nematodes obtained by elutriation of soil.

Saprobic nematodes were invariably found in large numbers within the polyethylene bags. *Hoplolaimus coronatus* Cobb and *Pratylenchus brachyurus* (Godfrey) were noticeable, particularly in samples buried longer than six months.

TABLE I

Field Survival of R. similis

	Fallow Soil								Infested Soil	
	Test 1 [1]					Test 2 [2]			Test 3 [3]	Test 4 [3]
Months	*31* [4]	*61*	*91*	*122*	*152*	*61*	*91*	*122*	*152*	*91*	*91*
1	+	+	+	+	+	+	+	+	+	+	—
2	0	+	+	+	+	+	+	+	+	+	—
3	0	0	0	+	+	+	+	0	+	+	—
4	0	0	+	+	+	0	0	+	0	+	—
5	0	0	0	+	0	0	0	0	0	+	—
6	0	0	0	0	0	0	0	0	0	— [5]	0
7	—	—	—	—	—	0	0	0	0	—	—
8	—	—	—	—	—	0	0	0	0	—	0
9	—	—	—	—	—	0	0	0	0	—	—
10	—	—	—	—	—	0	0	0	0	—	0
11	—	—	—	—	—	0	0	0	0	—	—
12	—	—	—	—	—	0	0	0	0	—	—

[1]) Samples in cheesecloth
[2]) Samples in nylon cloth and in polyethylene plastic
[3]) Samples in polyethylene plastic
[4]) Sample depth in cm
[5]) Bag torn, apparently by rodents

Code:
+ = *R. similis* present
0 = *R. similis* not found

Impenetrability of Polyethylene Plastic to Burrowing Nematodes

The foregoing tests suggested that *R. similis* cannot survive for more than six months in an environment that does not contain living roots of a host plant. Although polyethylene bags had been used in obtaining this information, the remote possibility existed that such material might be penetrated by this nematode, although apparently impenetrable by other species. In a greenhouse test, a 473 cc sample of chopped, infected roots and infested soil secured in polyethylene plastic was placed 2.5 cm from the bottom in each of five 30 cm wide clay pots containing

autoclaved soil. Four six-month-old grapefruit seedlings were then planted in each pot. Each month for five months one pot was examined, the roots of the four citrus plants incubated for a week, and the soil contained in the plastic bag elutriated. Thus if burrowing nematodes were capable of passing through the plastic, they could penetrate and subsequently could be recovered from the host citrus roots adjacent to the plastic bags.

It was found that *R. similis* populations decreased from a maximum of 70 for the first month to five for the fifth month. In no case did roots of the citrus seedlings yield burrowing nematodes, indicating that these nematodes did not penetrate the walls of their plastic enclosures.

Possible Selective Toxicity of Polyethylene Plastic to Burrowing Nematodes

Another possibility which might have influenced the results of the field tests was that the polyethylene plastic might be selectively toxic to burrowing nematodes despite the fact that numerous other types of nematodes maintained themselves and even thrived when confined in the bags.

The amount of plastic to which the nematodes contained in a 473 cc sample of soil would be exposed was calculated, shredded, and thoroughly mixed with burrowing nematode-infested soil in a 1.4 l capacity can in which a 22-month-old infected grapefruit seedling was growing. Controls received the same treatment, excluding incorporation of plastic with the soil. There were eight replicate plants per treatment. After nine months, the mean counts of burrowing nematodes were 218 ± 77 for the controls and 139 ± 37 for the plastic treatments. Although the difference between the means was great, it was not statistically significant. Thus it was concluded that the polyethylene used did not affect burrowing nematodes deleteriously.

Effect of Moisture on Survival

A final test in the greenhouse was made to determine whether varying moisture conditions would affect the longevity of burrowing nematodes. A 237 cc sample of chopped burrowing nematode-infected roots and infested soil in polyethylene was placed in each of thirty-two 3.8 l capacity cans of autoclaved soil. Eight cans were moistened and kept at field capacity (the maximum water-holding capacity of the soil), eight received no water, eight were kept on an alternating cycle from field capacity to dry conditions, while two healthy, two-month-old grapefruit seedlings were placed in each of the eight remaining cans that received normal watering. After six months the nematodes in the soil within each bag were counted while the roots of the grapefruit seedlings in the last treatment were incubated for a week and the evacuating *R. similis* counted.

The bags from the first three treatments, that were not associated with living citrus roots, yielded no burrowing nematodes. Three of the eight bags that had been placed in cans with two grapefruit seedlings were found intact and contained no burrowing nematodes, while the remaining five bags had been punctured by

the roots of the associated seedlings. The soil in one of these bags yielded one male and one female burrowing nematode, while the grapefruit roots growing through four of the bags contained *R. similis* populations. Thus burrowing nematodes had survived only where grapefruit roots penetrated the bags and provided a source of food for the imprisoned worms.

Effect of Two Temperatures on Survival

Continuing attempts to discover if burrowing nematodes would be capable of surviving for more than six months under various conditions, eight 473 cc samples of infected roots and infested soil were sealed in polyethylene bags and maintained in the laboratory at temperatures that averaged 24.4° C (15.0° C-33.9° C). An additional eight samples were placed in a refrigerator held at 1.1° C. After six months no *R. similis* were recovered from any of the samples, while both laboratory- and refrigerator-stored samples contained large numbers of saprobic nemas, notably of the genera *Rhabditis, Aphelenchus,* and *Cephalobus.*

DISCUSSION AND CONCLUSIONS

The first four tests made in the field indicated that burrowing nematodes confined in cheesecloth, nylon cloth, or polyethylene bags without a source of food at various depths down to 1.5 m perished in five to six months. This conclusion was not influenced by the placement of these bags either in infested groves or fallow soil. Additional tests in the greenhouse and laboratory conditions to study effects of temperature and moisture also supported this conclusion. There was no evidence that polyethylene plastic was toxic to or penetrated by *R. similis.*

It is possible that in these experiments, as well as in previous work, experimental conditions have not closely enough approximated those found in the field. Nematodes were always confined either in cloth, synthetic materials, or in containers which prohibit normal interchange of soil gases and moisture. Then, too, restricting the movement of the nematodes might possibly have had some bearing on their longevity. The confinement of nematodes in a soil mass containing large amounts of organic matter (as represented by the incorporated chopped roots) could have stimulated rapid growth of competing microorganisms which deleteriously affected burrowing nematode survival. Accordingly, under actual conditions *R. similis* can be present in the soil at depths of 2.4-3.1 m where there is little organic matter. Hence, the results of these tests, or others similarly designed, cannot be accepted as immutable proof. However, the general assumption on which the spreading decline eradication program is based (that the burrowing nematode cannot exist without food for two years) seems amply justified. These results show the necessity for keeping fumigated sites strictly fallow for at least six months.

RÉSUMÉ

Longévité de Radopholus similis *(Cobb) dans un sol sans hôte.*

Radopholus similis semble incapable de survivre pour plus de six mois dans les sols sablonneux de la Floride Centrale sans la présence de racines-hôtes. Des populations de cette espèce contenues

dans un mélange de racines et de sol de citrus et placées dans des sachets (d'étamine, de nylen ou de polyéthylène) enfouis a une profondeur de 0.3 à 1.5 m., disparurent en moins de six mois, tandis que dans les mêmes conditions *Hoplolaimus coronatus* Cobb, *Pratylenchus brachyurus* (Godfrey), et un grand nombre d'espèces saprophytes survécurent. Le polyéthylène ne fut pas toxique envers le *Radopholus similis*, et demeura imperméable à cette espèce dans des essais en serre. La présence de racines de citrus avec ou sans nématodes autour des sachets de polyéthylène enfouis contenant un mélange de racines et de sol de citrus infectés n'a eu aucune influence sur la longévité de *R. similis* contenu dans ces sachets. Tous les individus de l'espèce *R. similis* dans le sol et les racines contenus dans les sachets de polyéthylène n'ont pas survécu après une période de six mois ni à une température de laboratoire de 24.4° C ni à une température de 1.1° C. *Radopholus similis* dans un échantillon infesté et contenu dans des sachets de polyéthylène placés dans des pots de sol stérile maintenus à un température moyenne de 24.9° C pendant six mois ne survécurent pas lorsque les pots furent maintenus soit secs, soit humides, ou lorsque ceux-ci furent exposés à un cycle changeant de sec à humide.

REFERENCES

Birchfield, W. (1957). Observations on the longevity without food of the burrowing nematode. *Phytopathology* **47**, 161-162.

DuCharme, E. P. (1956). Sub-soil drainage as a factor in the spread of the burrowing nematode. *Proc. Fla. State Hort. Soc.* (1955) **68**, 29-31.

Malo, S. (1960). Comparative efficiencies of three methods for extracting nematodes from root and soil samples. *Plant Dis. Reptr* **44**, 217-219.

Seinhorst, J. W. (1956). The quantitative extraction of nematodes from soil. *Nematologica* **1**, 249-267.

Suit, R. F. & DuCharme, E. P. (1957). Spreading decline of citrus. *State Plant Board Fla.*, Vol. II, *Bul.* 11, 24 pp.

Tarjan, A. C., Simanton, W. A., & Russell, E. E. (1956). A labor-saving device for the collection of nematodes. *Phytopathology* **46**, 641-644.

Van der Vecht, J. (1950). Op planten parasiterende aaltjes. In: L. G. E. Kalshoven, *De plagen van de cultuurgewassen in Indonesië* **1**, 16-42.

Van der Vecht, J. & Bergman, B. H. (1952). Studies on the nematode *Radopholus oryzae* (van Breda de Haan) Thorne and its influence on the growth of the rice plant. *Gen. Agric. Res. Sta. Bogor Contrib.* **131**, 1-82.

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MAURICE BLOOD LINFORD

1901—1960

The death of Dr. Maurice Blood Linford on September 24, 1960, at Urbana, Illinois, brought a brilliant career to an untimely end. Trained as a plant pathologist, Dr. Linford contributed much through his work on plant diseases induced by fungi and viruses, but it was in plant nematology, a field in which he trained himself, that he made his major contributions.

Dr. Linford was born in Logan, Utah, on March 9, 1901. He was one of a family of six children, four of whom earned the Ph.D. degree. His bachelor's degree was earned in botany at Utah College in 1922 and his Ph.D. in plant pathology at the University of Wisconsin in 1927. After spending two years on a National Research Council fellowship, he became head of the department of plant pathology at the Association of Hawaiian Pineapple Canners experiment station (later the Pineapple Research Institute of Hawaii). In 1949 he was appointed Professor of Plant Pathology at the University of Illinois, where he remained until his death.

Helpfulness, politeness and gentle consideration characterized Dr. Linford's relations with his fellow men. Imaginative approach to problems, meticulous attention to detail and precision, both in technique and expression, characterized his work. His work on methods of observing soil fauna and on the feeding of nematodes is classic. His observations on potential agents of biological control of plant parasitic nematodes stimulated much work on this subject.

He was among the few, thirty years ago, to recognize the value of proper design of field experiments and statistical evaluation of results and to make use of these precise techniques. While great imagination led to many ideas, he did not hesitate to discard those which failed in the rigorous tests to which he subjected them.

Dr. Linford is survived by a son and daughter, his mother, a sister, four brothers and three grandchildren. His wife preceded him in death in 1949.

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BUCHBESPRECHUNG

Meyl, A. H.: Freilebende Nematoden. In: Die Tierwelt Mitteleuropas. Herausgegeben von P. Brohmer, P. Ehrenberg und G. Ulmer, I, Lief. 5a. Mit 54 ganzseitigen Tafeln, 164 S. Verlag Quelle und Meyer, Leipzig. Preis 20.— DM.

Bei der schnellen Entwicklung der Nematodenforschung und den zahlreichen Neubeschreibungen in den letzten 20 Jahren war eine Neubearbeitung der Nematoden in deutscher Sprache dringend erforderlich. Diese ist nunmehr im Rahmen des Werkes „Tierwelt Mitteleuropas" erschienen. Verfasser beschränkt sich hauptsächlich auf die freilebenden Erd- und Süsswassernematoden, soweit sie bis zum 1. Juni 1959 aus Europa bekanntgeworden sind. Rein marin lebende Arten werden nicht erwähnt. Nach einem kurzen Abriss über Morphologie und Entwicklung der Nematoden und einigen technischen Hinweisen folgt ein Schlüssel der Abkürzungen, ohne den der Leser sich nicht zurechtfinden könnte. Dieser enthält gleichzeitig die entsprechenden Bezeichnungen in englischer Sprache, sodass er auch für den der deutschen Sprache weniger mächtigen Forscher von Wert ist. So gewinnt das Buch auch über die deutschen Sprachgebiete hinaus an Bedeutung. Die Bestimmungstabellen der Gattungen nehmen keine Rücksicht auf die Familienzugehörigkeit, sondern sind nach den am besten erkennbaren Merkmalen aufgestellt. In den Tabellen zur Artbestimmung werden die Gattungsmerkmale jeweils vorangestellt, dann folgen die für die Artbestimmung notwendigen Kennzeichen. Auch ökologische Hinweise und Angaben über das Vorkommen und die Verbreitung fehlen nicht. Bei jeder Art wird auf die entsprechende Literatur verwiesen, die in einem besonderen Verzeichnis mit 331 Nummern zusammengestellt ist. In vielen Fällen wird man auf die Benutzung dieser Spezialliteratur nicht verzichten können, zumal sich die Abbildungen (Strichzeichnungen) auf die wichtigsten Merkmale beschränken. Dennoch sei hervorgehoben, dass der Verfasser in zäher Kleinarbeit auf knappem Raum eine Fülle von Einzelergebnissen zusammengetragen und sie mit grosser Sorgfalt übersichtlich verarbeitet hat. So entstand ein Bestimmungsbuch, das auch vom Nematologen gern zur Hand genommen wird. Wir wünschen dem Buch eine weite Verbreitung.

Goffart (Münster)

Nematologica 6 (1961) : 177-180. Leiden, E. J. Brill

THE INFLUENCE OF RESISTANT POTATO VARIETIES ON THE SOIL POPULATION OF *HETERODERA ROSTOCHIENSIS* WOLL.

BY

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A field experiment in sandy soil near Wageningen has shown that growing of resistant potato varieties decreases the degree of infestation with *Heterodera rostochiensis* more rapidly than growing immune crops (beans). After growing resistant varieties for 6 years the soil was still noticeably infested. There was an indication that after three generations of such varieties the degree of infestation did not continue to decrease, but that it was maintained at a definite level. This level was independent of the initial degree of infestation. At this level the resistant crop was not damaged appreciably. It is considered impossible to eradicate the parasite completely by growing resistant varieties.

The clone CPC 1673 of *Solanum tuberosum* subsp. *andigena* has been almost the only source of resistance to *Heterodera rostochiensis* Woll. used in potato breeding in the Netherlands. This clone and the resistant hybrids obtained from it produce root diffusates equally as effective in inducing larvae of *H. rostochiensis* to hatch as those of susceptible plants and the larvae invade the roots of resistant potatoes as freely as those of susceptible ones. Only few cysts develop on the roots of resistant plants but these may contain many eggs. When brought once more together with resistant plants, only few larvae of these cysts develop into mature females (Huijsman, 1957). Various authors have already shown that high populations of the nematode are reduced more rapidly by growing resistant hybrids, presumably because they produce active roots diffusate, than by fallow or immune crops (Cole & Howard 1959, Huijsman 1957, Rothacker & Stelter 1959). The results of a field experiment at Wageningen, started in 1954, suggest however, that this is not valid for low populations and that on resistant hybrids the nematode can maintain a low population density.

In an experiment on a fairly uniformly infested field of sandy soil at Wageningen resistant potatoes were grown continuously on five plots from 1954 and two from 1955; beans (*Phaseolus* sp.) were grown on five other plots from 1954 onwards and on three more from 1955. The population density of *H. rostochiensis* in the five plots brought into the experiment in 1955 was almost ten times as high as that of those brought in in 1954. In 1954 insufficient tubers were available to plant all the experimental plots with one clone and tubers of many different clones were planted. Subsequently the number of clones was decreased. In 1958 for the first time, tubers of one resistant variety only were planted.

In the autumn of each year population densities of the parasite were determined. Bulk soil samples of approximately 3 kg were taken from each plot by combining 80 subsamples and no soil was collected from areas less than half a meter from

the edges. The bulk samples were dried and sieved and the number of cysts and encysted eggs and larvae were determined from 400 g of each (Huijsman 1957). By puncturing eggs with questionable contents the presence or absence of larvae was established. Whether the larvae counted in this way were infective cannot be said. However, according to Den Ouden (personal communication) the number of hatchable larvae is about 80% of the number of eggs and larvae counted as

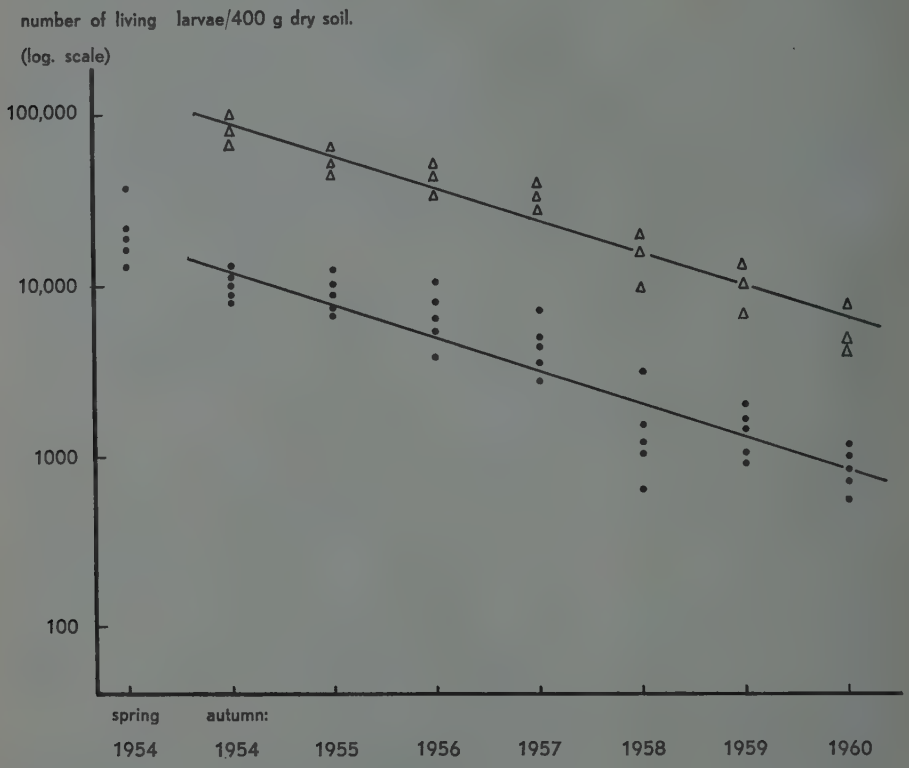

Fig. 1. Changes in the potato root eelworm population in terms of the number of living eggs per 400 g dried soil when an immune crop (beans) is grown continuously.
△——△: Heavily infested plots (1955); ●——●: moderately infested plots (1954).

described above, even in populations from soil which had not carried potatoes for 5 years.

The results of the experiment are presented graphically in figures 1 and 2. Fig. 1 shows a more or less rectilinear relation between the logarithm of the number of living larvae per unit weight of soil and time, when beans, an immune crop, are grown on infested soil for a number of years. In 5 years the population decreased to about an eighth of the original density, a decline of about 33% per year.

Fig. 2 shows the change in the degree of infestation of the plots when resistant

potatoes were grown for 6 consecutive years. At first the population decreased more rapidly than when immune crops were grown. However, the data suggest that, after growing resistant potatoes for 3 or 4 years, no further decrease of the degree of infection took place. It continued to fluctuate around a value of some hundreds of living larvae per 400 g of dry soil.

This may be explained in the following way. A few cysts are always found on the roots of resistant plants raised in heavily infested soil. This small number of

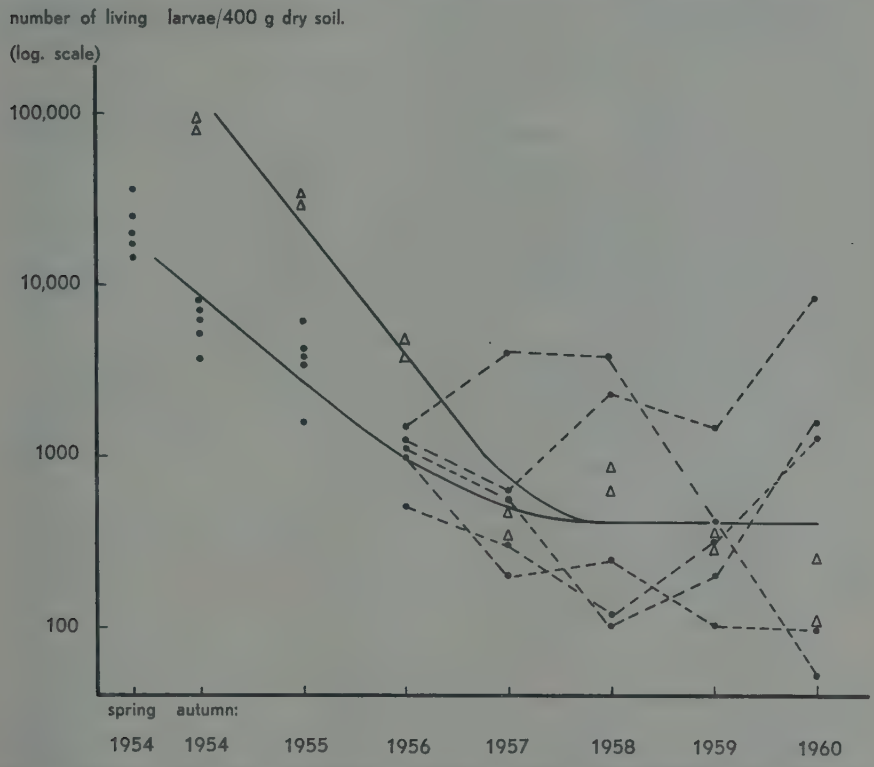

Fig. 2. Changes in the potato root eelworm population in terms of the number of living eggs per 400 g dried soil when resistant potato varieties were grown continuously.
△——△: Heavily infested plots (1955); ●——●: moderately infested plots (1954).

cysts might also be produced at low levels of infestation, as a much higher percentage of invading larvae would then be able to occupy places in the roots favourable for the development of a female. Actually there is no reason to expect that the number of eggs produced by these females could not be higher than the number of larvae invading the roots when the initial population density of the soil is very low. In this way a more or less constant population density would be maintained by growing resistant varieties continuously.

However, from 1957 on, the differences in the egg densities between the plots

However, from 1957 on, the differences in the egg densities between the plots which carried resistant potatoes were much greater (ratio between lowest and highest count in 1960 : 1 : 120) than those between the plots which carried beans (ratio between lowest and highest count in 1960 : 1 : 2.2). This cannot be ascribed to the counts in the first group being very low. In 1960 the mean numbers of eggs per unit weight of soil were about the same for both treatments. Fairly high numbers of eggs in some of the resistant-potato plots could be explained by the presence of a race of the eelworm, which multiplies well on resistant potatoes, race B (Dunnett 1957). These high numbers would have originated from mixing subsamples containing many eggs with subsamples containing few eggs.

To test this hypothesis 25 samples were taken in the resistant-potato plot with the highest egg density. The numbers of eggs found in these samples ranged from 650-13,000 per 400 g of soil with a mean of 5100 eggs. If race B was present in the plots of resisters with high egg-counts, multiplication during six years would have resulted in the presence of at least 100,000 eggs per 400 g of soil. Moreover the population trends in the plots were not consistently the same (see Fig. 2). In some cases high population densities decreased, low ones increased considerably, when resistant potatoes were grown. If race B was present in some of the plots the population densities in these would have kept rising until a very high level was reached. Therefore it seems unlikely that race B occurs in the resistant-potato plots.

The differences in population density between these plots are probably due to differences in susceptibility between plants of the clone used in the experiment and the distribution of more or less susceptible plants in the plots.

The results of this experiment suggest that growing resistant varieties of potatoes for many consecutive years will not lead to disappearance of the parasite, but that a small number of very irregularly distributed potato-root eelworms will remain in the field.

I am much indebted to Dr. J. W. Seinhorst for advice and helpful criticism.

ZUSAMMENFASSUNG

Der Einfluss resistenter Kartoffelsorten auf die Bodenpopulation von Heterodera rostochiensis *Woll.* Ein Feldversuch in Sandboden nahe Wageningen ergab, dass der Anbau resistenter Kartoffelsorten den Befallsgrad mit *Heterodera rostochiensis* stärker vermindert als ein Anbau immuner Pflanzen. Nach dem Anbau resistenter Sorten für 6 Jahre war der Boden noch beachtlich verseucht. Es bestehen Anzeichen dafür, dass nach dreimaligem Anbau solcher Sorten der Befallsgrad nicht weiter absinkt, sondern dass er sich auf einer bestimmten Höhe hält. Diese Höhe war unabhängig von dem Anfangsgrad der Verseuchung. Auf dieser Höhe wurde die resistente Frucht nicht bemerkenswert geschädigt. Es wird für unmöglich gehalten, den Parasiten durch Anbau resistenter Sorten vollständig zu tilgen.

REFERENCES

Cole, C. S. & Howard, H. W., (1959). The effect of growing resistant potatoes on a potato root eelworm (*Heterodera rostochiensis* W.) population. *Nematologica* **4**: 307-316.

Dunnett, J. M., (1957). Variation in pathogenicity of the potato root eelworm (*Heterodera rostochiensis* Woll.) and its significance in potato breeding. *Euphytica* **6**: 77-89.

Huijsman, C. A., (1957). Veredeling van de aardappel op resistentie tegen *Heterodera rostochiensis* Wollenweber. *Dissertatie Landbouwhogeschool, Wageningen.*

Rothacker, D. & Stelter, H., (1959). Beiträge zur Resistenzzüchtung gegen den Kartoffelnematoden (*Heterodera rostochiensis* Wollenweber). IV. Das Verhalten von resistenten Bastardklonen aus der Kreuzung zwischen *S. tuberosum* subsp. *andigena* mit *S. tuberosum* auf nematodenverseuchten und nematodenfreien Flächen. *Züchter* **29**: 241-251.

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STUDIES ON THE CULTURING AND PARASITISM OF PLANT-PARASITIC NEMATODES, IN PARTICULAR *DITYLENCHUS DIPSACI* AND *APHELENCHOIDES RITZEMABOSI* ON ALFALFA TISSUES [1])

BY

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Ditylenchus dipsaci and *Aphelenchoides ritzemabosi* reproduced rapidly on alfalfa callus tissue grown on a nutrient agar medium containing 2,4-dichlorophenoxyacetic acid (2,4-D). Cultures inoculated with 50 nematodes and kept at 22° C contained 40,000 to 80,000 individuals after two months. *Pratylenchus zeae* and *P. penetrans* reproduced well and *Tylenchorhynchus capitatus* and *Hoplolaimus coronatus* slowly on callus. Four of these species were maintained alive in such cultures for two years without subcultures.

The histopathology of *D. dipsaci* and *A. ritzemabosi* on alfalfa seedlings grown in soil, seedlings grown aseptically on nutrient agar medium, and callus tissue grown aseptically on nutrient agar medium plus 2,4-D was examined. *D. dipsaci* penetrated directly through the epidermis in the bases of cotyledons of alfalfa seedlings in soil and formed cavities in cortical parenchyma within 12 hours of inoculation. Infected stems were noticeably swollen at 36 hours after inoculation. Many parenchyma cells were destroyed and others distorted in galled tissue, particularly in young, actively growing shoots. Only cavities were formed in roots of infected seedlings growing on agar medium with no galling; shoot galls were identical with those of infected plants in soil. *A. ritzemabosi* fed primarily as an ectoparasite on shoots of alfalfa seedlings inhibiting apical growth and resulting in severely stunted and malformed plants, but a few lived in the mesophyll of cotyledons. Both nematode species fed and reproduced in the loose parenchyma of callus tissue, with the nematodes moving from the callus to the agar and test tube walls in older cultures.

Sugars, organic acids and amino acids were examined in alfalfa tissues as used in the histological studies. Neither nematodes nor the conditions under which plants or tissues were grown affected qualitatively the sugars or organic acids, and affected only two of the 23 major amino acids identified. Gall tissue of plants infected by *D. dipsaci* and all callus tissue samples contained free tryptophane whereas healthy plants or those parasitized by *A. ritzemabosi* did not. Tissue parasitized by *A. ritzemabosi* contained abundant free tyrosine whereas nematode-free or *D. dipsaci*-infected tissues contained little or none.

Various workers have made use of plant tissue cultures in the study of plant-parasitic nematodes. Tyler (1933) used tomato roots growing aseptically on a nutrient agar medium to study the reproduction of *Meloidogyne* sp., and Mountain (1955) used root cultures of corn, tobacco and red clover to study the pathogenicity of *Pratylenchus minyus* Sher & Allen. *Ditylenchus destructor* Thorne was cultivated aseptically on undifferentiated tissues of potato, carrot, clover and

[1]) Most of this work was completed while the author was a National Science Foundation Post-doctoral Fellow in the Nematology Department at Rothamsted Experimental Station, the remainder at the University of Maryland.

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tobacco, and also on cultures of 37 different fungi growing on potato dextrose agar medium (Darling, Faulkner & Wallendal, 1957). Peacock (1958) used root cultures of *Lycopersicon peruvianum* L. to study resistance to *Meloidogyne incognita* (Kofoid & White). *Heterodera rostochiensis* Woll. matured, but did not reproduce on tomato roots in culture (Widdowson, Doncaster & Fenwick, 1958).

Early work on histopathology and symptoms on plants attacked by *Ditylenchus dipsaci* (Kühn) and *Aphelenchoides ritzemabosi* (Schwartz) were summarized by T. Goodey (1935). *D. dipsaci* was parasitic mainly in the intercellular spaces of the cortical parenchyma in stems of various plants. Nematodes were thought to secrete pectinases, which dissolved the middle lamellae between cells. When infected tissues were actively growing, hypertrophy and hyperplasia occurred in cells surrounding the nematodes with an increase in intercellular spaces as the galls enlarged. *A. ritzemabosi* fed ecto- or endoparasitically, depending on the particular plant or plant part attacked. Older leaves of susceptible chrysanthemum plants were entered through the stomata and the nematodes lived in the intercellular spaces of the spongy mesophyll. The first reaction to the nematodes was that the chloroplasts turned yellow, after which the protoplasm aggregated at the cell centers and the cells finally turned brown. Nematodes rarely penetrated between palisade mesophyll cells, which became discolored later than spongy mesophyll cells. Ectoparasitic feeding also occurred on embryonic tissues of tightly rolled buds of young chrysanthemum plants retarding bud growth and resulting in small, distorted and crinkled foliage.

Quanjer (1927), T. Goodey (1929), and J. B. Goodey (1948) discussed the cause of gall formation in plants infected by *D. dipsaci.* Some considered pectinase activity to be of prime importance and that this enzyme had to dissolve the middle lamellae between cortical parenchyma cells before gall formation was initiated; other irritants secreted by the nematodes stimulated formation of the actual gall. T. Goodey (1935) observed that galls formed only when infected tissues were actively growing. After the discovery of plant growth regulators J. B. Goodey (1948) suggested that such substances might be secreted into the plant tissues by the infecting nematodes causing the hypertrophy and hyperplasia resulting in gall development. Nolte & Köhler (1952) found that homogenates of *D. dipsaci* and extracts of nematode-infected rye promoted growth of sunflower seedlings.

The interactions of nematodes and the mineral nutrition of plants have been studied by Oteifa (1952, 1953) and Dropkin & King (1956), and the effects of nematodes on the organic composition of plants by Owens & Novotny (1960).

The present work sought (1) do develop a means of propagating plant-parasitic nematodes in large numbers monoxenically using plant tissue cultures, (2) to study the histopathological effects of *D. dipsaci* and *A. ritzemabosi* on alfalfa (lucerne, *Medicago sativa* L.) plants growing in soil, growing aseptically on a nutrient agar medium and growing aseptically as callus tissue on a nutrient agar medium, and (3) to study the effects of parasitism by these two nematodes on

these variously-grown alfalfa tissues on the qualitative analysis of free sugars and organic acids and both free and bound amino acids in tissues of these plants. It was hoped to correlate biochemical changes in the plant tissues with histopathological changes caused by nematode parasitism.

MATERIALS AND METHODS

Culturing

Undifferentiated tissues derived from alfalfa seedlings were cultured on a nutrient agar medium and were used as the substrate for propagating plant-parasitic nematodes. The composition of the nutrient agar medium was: $Ca(NO_3)_2.4H_2O$, 500 mg; KNO_3, 125 mg; $MgSO_4.7H_2O$, 125 mg; KH_2PO_4, 125 mg; H_3BO_3, 1 mg; $FeSO_4.7H_2O$, 3.3 mg; $MnCl_2.4H_2O$, 3.6 mg; $ZnSO_4.7H_2O$, 0.44 mg; $Na_2Mo_2O_4$, 0.25 mg; $CuSO_4.5H_2O$, 0.16 mg; glycine, 0.3 mg; nicotine acid 0.05 mg; pyridoxine. HCl, 0.01 mg; thiamine. HCl, 0.01 mg; 2,4-dichlorophenoxyacetic acid (2,4-D), 2 mg; sucrose, 20 gm; agar, 15 gm; coconut milk, 150 ml; distilled water, to 1000 ml.

Later a different nutrient agar medium was used which stimulated more rapid growth of alfalfa callus and nematode reproduction than the medium above. This medium was used by Hildebrandt, Riker & Duggar (1946) for culturing tobacco tissues and has the following formula: Na_2SO_4, 800 mg; $Ca(NO_3)_2.4H_2O$, 400 mg; $MgSO_4.7H_2O$, 180 mg; KNO_3, 80 mg; KCl, 65 mg; $NaH_2PO_4.H_2O$, 33 mg; $MnSO_4.4H_2O$, 4.5 mg; $ZnSO_4.7H_2O$, 6 mg; H_3BO_3, 0.375 mg; KI, 3 mg; ferric tartrate, 40 mg; glycine, 3 mg; thiamine. HCl, 0.1 mg; Ca pantothenate, 2.5 mg; alpha-naphthaleneacetic acid (NAA), 0.1 mg; 2,4-D, 2 mg; sucrose, 20 gm; agar, 15 gm; coconut milk, 150 ml; distilled water, to 1000 ml.

The medium was placed in 1 × 6-in. test tubes (15 ml/tube), tubes were capped with aluminum foil, autoclaved and the medium solidified as slants. Alfalfa seeds (var. DuPuits) were surface-sterilized by immersion in conc. H_2SO_4 for 30 minutes, drained of the excess acid and all the acid removed by several copius washes in sterile distilled water. The seeds were then placed on 2% water agar to germinate and, when about 2.6 cm long, the seedlings were transferred aseptically to the slants of nutrient agar, two to six seedlings per slant. The alfalfa tissue cultures were propagated for 2-3 weeks before surface-sterilized nematodes were introduced.

Nematodes from infected plant parts or infested soil were surface-sterilized by passing them singly with a needle through five or six baths of a solution of 20 ppm malachite green plus 1000 ppm streptomycin sulfate, contained in sterile Syracuse watch glasses. Nematodes were in contact with these solutions for three to four hours before being transferred directly by needle to the agar slants containing plant tissue cultures. The aluminum foil caps were then sealed onto the tubes with cellophane tape and the cultures incubated at 25° C in the light or dark.

Histopathology

The *A. ritzemabosi* used came from chrysanthemum at Harpenden, England, and the *D. dipsaci* from alfalfa near Raleigh, North Carolina. Alfalfa tissues without and with nematodes were grown as (1) seedlings in soil, (2) aseptic seedlings on nutrient agar lacking 2,4-D, and (3) aseptic callus tissue on nutrient agar containing 2,4-D. Coconut milk was not added to the agar medium used in these and the biochemical studies because unknown organic substances might affect the analyses for excreted compounds. Omitting coconut milk had little effect on nematode activity, although callus tissue grew slightly more slowly.

Alfalfa seedlings were grown in small flats of soil, 30 × 45 × 10 cm, to study the initial stages of penetration of plants by *D. dipsaci.* Seedlings began emerging 2 days after planting when 28,000 nematodes in water suspension were distributed evenly over the seedlings and soil. The flat was covered with a plastic sheet to maintain a high humidity and was kept at 27° C under fluorescent light. Thirty-six shoots picked at random at each time interval were killed and fixed at 6, 12, 18, 24 and 36 hours after nematode inoculation.

Later stages of parasitism of alfalfa seedlings growing in soil and on agar medium by both *D. dipsaci* and *A. ritzemabosi* were studied separately. Seedlings were grown in 15 ml (1 × 6-in.) test tubes filled with soil in the greenhouse. When the seedlings were 1.3 cm tall, 200 specimens of one or the other nematode species in water suspension was added to each tube. The tubes were then placed in a plastic bag for one week to maintain a high humidity. Slants of nutrient agar medium with or without 2,4-D and planted one week previously with six alfalfa seedlings per tube were inoculated with 50 surface-sterilized individuals of one or the other nematode species. Seedlings or callus tissue were killed and fixed at 2, 5, 10, 20, 30, 45 and 60 days after inoculation. Seedlings in tubes without nematodes served as controls.

Alfalfa seedlings growing in soil in flats and parasitized by *A. ritzemabosi* were also collected for histopathological studies. Two months after inoculation with nematodes many of these plants were severely stunted and deformed. Healthy and *Ditylenchus*-galled alfalfa tissues from a natural field infestation were collected to compare with similar tissues grown under controlled conditions.

Plant tissues were fixed in CRAF III (30 ml 1% chromic acid, 20 ml 10% acetic acid, 10 ml 40% formalin, 40 ml water) (Sass, 1958). Before killing, older callus tissues were dipped for a few seconds in liquid 2% agar at 40° C to coat them and to prevent the tissues from falling apart during processing for sectioning. Tissues were dehydrated and cleared in an ethanol-xylene series, embedded in 54° C m.p. paraffin and sectioned at 10 or 20 microns, stained with safranin and fast green and mounted in canada balsum.

Biochemical

Alfalfa seedlings were grown in flats of soil in the greenhouse, as seedlings

on 40 ml of nutrient agar medium lacking 2,4-D in 250 ml Erlenmeyer flasks, or as callus on the agar medium plus 2,4-D in Erlenmeyer flasks (Fig. 1, A, B). Plants in soil in flats were inoculated with nematodes when 1.25 cm tall, or in flasks about two weeks after transfer of seedlings to the agar medium. Flats of plants were inoculated with about 6,000 *D. dipsaci* or 15,000 *A. ritzemabosi* in water suspension. Seedlings or callus in flasks were inoculated with nematodes indirectly by transferring plant tissues from month-old cultures in test tubes inoculated initially with 50 nematodes per tube, so the number of nematodes added to each flask was not known. Plants in flats were harvested for chemical analyses two months after inoculation and those in flasks 36 days after inoculation. Only obviously parasitized tissues from inoculated plants growing in soil and comparable tissues from non-parasitized plants were used in the analyses. Similarly, inoculated and nematode-free alfalfa tissues growing on agar medium in flasks were compared.

Freshly-harvested plant tissues were macerated in a blender for 10 min. in 50 ml of 80% ethanol at room temperature (Block, Durrum & Zweig, 1958). Samples (five gm fresh weight) of tissues from seedlings growing in soil, or all the tissues in flasks, which ranged from 1.0-1.7 gm, were used. Samples (five gm) of healthy and *Ditylenchus*-galled alfalfa from the field were also analyzed. After removing the plant tissue, the agar medium in each flask was extracted with 50 ml of 80% ethanol and these extracts were treated in the same manner as plant extracts. Each extract was filtered through a Buchner funnel and the blendor flask and residue rinsed with another 35 ml of 80% ethanol. The combined extract and rinse were evaporated to dryness in a rotary evaporator at less than 60° C.

Massed specimens of nematodes were also analyzed chemically. *D. dipsaci* was separated from cultures on alfalfa callus tissue with Baermann funnels. *A. ritzemabosi* was obtained from infected dried chrysanthemum leaves by the water mist technique (J. B. Goodey, 1957). Nematodes were concentrated initially by settling and finally by centrifugation at about 500 xG. Massed nematodes were washed free from soluble substances and micro-organisms, as described by Krusberg (1960), and macerated in 80% ethanol with powdered glass in a mortar. These macerates were treated exactly as the plant samples.

Plant and nematode residues were then hydrolyzed at 100° C for 18 hours in sealed glass digestion tubes containing the dry residue and 10 ml of 6N HCl to assay the amino acids in the proteins. The residue after hydrolysis was removed by filtration, rinsed with water, and the combined filtrates evaporated and treated thereafter as the ethanolic extracts.

All the evaporated extracts were then purified. The residue from the evaporated extract was dissolved in 15 ml of distilled water and any undissolved material filtered out. Solutions were adjusted to pH 7.0 $\pm$ 0.1 with HCl or NH_4OH and then passed through columns of ion exchange resins to remove ions that would interfere with paper chromatographic separations of the components ac-

cording to the method of Thompson, Morris & Gering (1959). Zeo-Karb 225 resin was used instead of Dowex 50-X4 (200-400 mesh) resin. All operations were at room temperature (19-24° C). The NH_4^+ form resin was not eluted with 6N HCl because no amino acids were detected in this eluate. The ion exchange columns were arranged one above the other so that the effluent from one dripped into the one below as follows: ZeoKarb 225 NH_4^+ form column (to adsorb basic amino acids) above a Zeo-Karb 225 H^+ form column (to adsorb neutral and acidic amino acids), and the latter above an Amberlite IRA-400 $CO_3^=$ form column (to adsorb organic acids) (Bryant & Overell, 1953); the effluent contained the sugars.

The eluates from the NH_4^+ and H^+ form resins were combined and the eluate from the $CO_3^=$ form resin and the effluent were kept separate giving three fractions, an amino acid, an organic acid and a sugar fraction. These solutions were evaporated to dryness in a rotary evaporator at less than 60° C and the residues washed out in a few ml of 10% isopropanol. Each isopropanol solution was further concentrated to a few tenths of a ml in small dishes on a warming table at less than 60° C. These fractions were analyzed separately by paper chromatography.

A portion or the entire amount of the final isopropanol solution was spotted on a 46 × 57-cm sheet of Whatman No. 1 chromatography paper for 2-dimensional chromatography (Block *et. al.* 1958). The papers were developed by the method of Pleshkov (1959). The first directional solvent was phenol : water (3 : 1) in an ammonia atmosphere plus a few mg of KCN in water in a dish in the chromatography cabinet to prevent the phenol oxidizing. The second directional solvent was n-butanol: acetic acid: water (9 : 1 : 2.9). After each development the papers were dried in a chromatography oven at 40° C. Papers spotted with organic acids were also steamed to remove acetic acid residues. Papers were sprayed according to Block *et al.* (1958) to identify the following types of compounds: for sugars, 3% anisidine hydrochloride in n-butanol; for organic acids, 0.04% chlorophenol red in 95% ethanol at pH 7.0; and for amino acids, 0.3% ninhydrin in 95 % ethanol. The organic acids were further identified using the spray reagents for color reactions after Buch, Montgomery & Porter (1952). In addition to the position on the chromatograms and the reaction with ninhydrin, Ehrlich's reagent was used to identify tryptophane (Block *et al.* 1958). Only qualitative analysis of these compounds was attempted. Identifications were based mainly on positions of spots on the papers and the color reactions with the spray reagents comparing the chromatograms of the unknown compounds with chromatograms of known ones.

RESULTS

Culturing

Tissues of carrot, sweetpotato, tobacco, tomato and soybean, in addition to alfalfa were tried as substrates for nematodes. Initial attempts, begun in North

Carolina in 1958, were made with *Ditylenchus dipsaci, Pratylenchus zeae* Graham, *Tylenchorhynchus capitatus* Allen, *Hoplolaimus coronatus* Cobb, *Helicotylenchus nannus* Steiner, *Ditylenchus triformis* Hirschmann & Sasser, *Meloidogyne incognita acrita* Chit. and a *Trichodorus* sp. *D. dipsaci* and *P. zeae* readily became established on alfalfa callus and reproduced rapidly, but reproduced slowly or not at all on tissue cultures of other plants. *T. capitatus* and *H. coronatus* reproduced slowly on alfalfa callus and not at all on other plant cultures. The remaining species did not reproduce on any tissues. In England *Aphelenchoides ritzemabosi* was cultured and reproduced more rapidly on alfalfa callus than any other nematode in culture. Recently *Pratylenchus penetrans* (Cobb) was readily cultured similarly. Fig. 1, B shows some callus cultures.

D. dipsaci, P. zeae, T. capitatus and *H. coronatus* remained alive without subculture for 2.5 years. *A. ritzemabosi* reproduced very rapidly on alfalfa callus causing rapid decline and partial disintegration of the tissue cultures. Sealing the tubes with aluminum foil held by cellophane tape allowed enough gas exchange, but prevented most water loss even after 2.5 years. Thus it should be satisfactory to subculture *D. dipsaci* and *P. zeae* annually and *A. ritzemabosi* every six months. *D. dipsaci* cultured on alfalfa callus for two years remained able to cause galls and reproduce on alfalfa growing in soil.

Nematodes reproduced rapidly on alfalfa callus tissue. The nematodes in a few flasks from the biochemical studies containing *D. dipsaci* and *A. ritzemabosi* were counted. Flasks inoculated with 50 nematodes contained, after two months, the following numbers: *A. ritzemabosi* on seedlings 19,000, on callus 77,000; *D. dipsaci* on seedlings 28,000, on callus 42,000. Fresh weight of the plant tissue was between 1.0 and 1.7 gm per flask. The reproduction of the other species in culture was not measured.

All three nematode species that reproduced rapidly on alfalfa callus broke down the agar medium as the cultures aged. Test tube cultures heavily infested with *D. dipsaci* were stored upright and the slanted agar partially liquefied, became mushy and slid to the bottoms of the tubes in year-old cultures.

Endo (1959) reported that *P. zeae* did not reproduce on alfalfa growing in soil. This was confirmed and although a few roots were penetrated no reproduction occurred in six months on seedlings growing on agar medium. However, a year-old culture of *P. zeae* on callus in a test tube inoculated initially with 50 specimens contained 19,000 nematodes.

Histopathological

Initial Stages of Infection of Alfalfa by D. dipsaci

6 hours — Nematode damage to alfalfa shoots was visible six hours after inoculation. Nematodes 1) invaded between the tightly-packed members of the terminal bud and penetrated directly downwards into the parenchyma subtending the cotyledon axils, 2) penetrated laterally into the cotyledons slightly above the axil usually opposite a vascular bundle, and 3) attacked embryonic leaves or the

shoot apex. The first indication of damage was that affected parenchyma cells, stained with safranin and fast green, appeared purplish whereas unaffected cells stained green. Nucleoli in normal cells stained red, but nuclei and nucleoli in affected cells were slightly enlarged and stained purplish. Affected cells were slightly enlarged and sometimes seemed to be separating from one another.

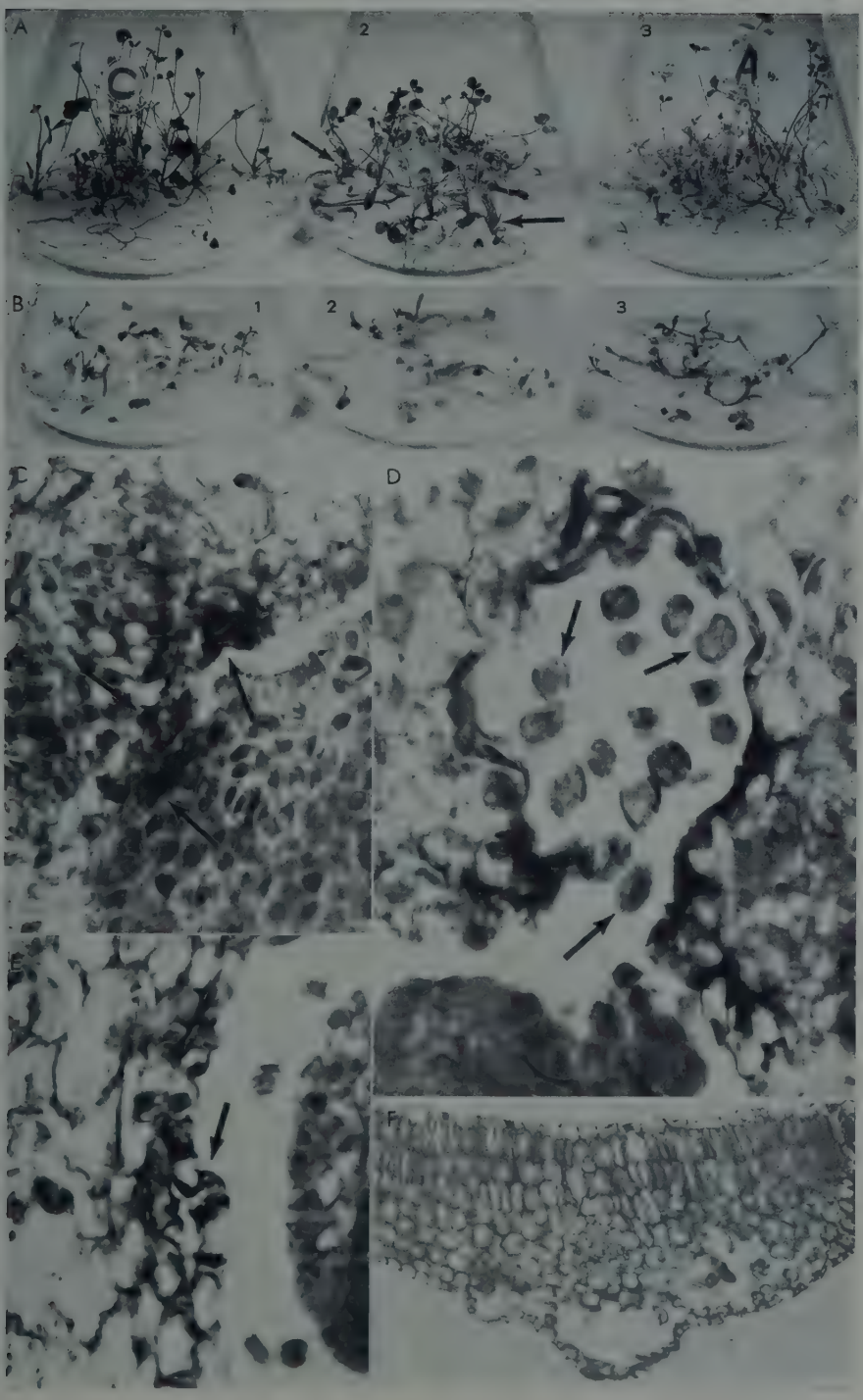
In some discolored areas of the cotyledon base one or two epidermal and a few underlying cortex cells had been punctured by nematodes, were collapsed and empty, or the area of broken cells and cell remnants appeared thick, amorphous and stained almost black. Some cell walls were deformed, but others appeared normal; however, the contents of affected cells were always discolored (Fig. 1, C). Discoloration extended two to six cells into the cortex underlying the point at which the epidermis had been attacked, both where epidermal cells had been punctured and where they were only discolored. Cytoplasm in cortical parenchyma cells adjacent to punctured cells was highly vacuolated, granular and stained purplish. Nuclei in these adjacent intact cells were often barely visible because of the deep purplish staining of the cytoplasm; these nuclei were enlarged and stained almost black, and the nuclear membranes indistinct. Nucleoli in these nuclei were stained deep red and indistinct membranes. Walls of adjacent affected cells were darkly stained, swollen and obscure.

The cytoplasm in epidermal cells of affected embryonic leaves and in a few adjacent parenchyma cells was granular, vacuolate and purplish, nuclei were enlarged and nucleoli very large and stained bright red; nuclear and nucleolar membranes were often indistinct. These cells had not yet enlarged or separated.

12 hours — At 12 hours after inoculation, nematodes had already penetrated and formed a cavity in one cotyledon (Fig. 1, D) containing about 15 nematodes. Stem galling had not begun, although some of the intact cortical cells adjacent to the cavity were damaged, as indicated by slight enlargement of cells, nuclei and nucleoli, purplish coloration of the protoplasm, increased vacuolation and thickening and dark-staining of cell walls. The cavity was lined with a thick layer of darkly-stained substance, presumably residue from cells destroyed during formation of the cavity by the nematodes. The cavity extended both upward into the cotyledon and downward into the cortex of the stem from the point of nematode penetration near the cotyledon base.

Tissue damage at 12 hours was more pronounced, although similar to that at

Fig. 1. Alfalfa parasitized by *D. dipsaci* and *A. ritzemabosi*. A.) Sterile seedlings growing on nutrient agar medium in flasks; 1, control; 2, parasitized by *D. dipsaci*, note galls (arrows); 3, parasitized by *A. ritzemabosi*. × ½. B.) Callus tissue growing on nutrient agar medium plus 2,4-D in flasks; 1, 2 and 3 parasitized as in A. × ½. C-F.) Seedlings inoculated with *D. dipsaci*. C.) Cross section through the region of the cotyledon base 6 hours after nematode inoculation. Note the necrotic epidermal cell and area of discolored cells (arrows). × 394. D.) Cross section of an infected cotyledon just above the axil 12 hours after nematode inoculation. Nematodes (arrows) in cavity. × 394. E.) Cross section of a cotyledon base a few mm above the axil 12 hours after nematode inoculation. Note bulging epidermal cells (arrow) and dark-stained, thickened cell walls in the attacked area. × 376. F.) Cross section of an infected cotyledon lamina 36 hours after nematode inoculation. × 93.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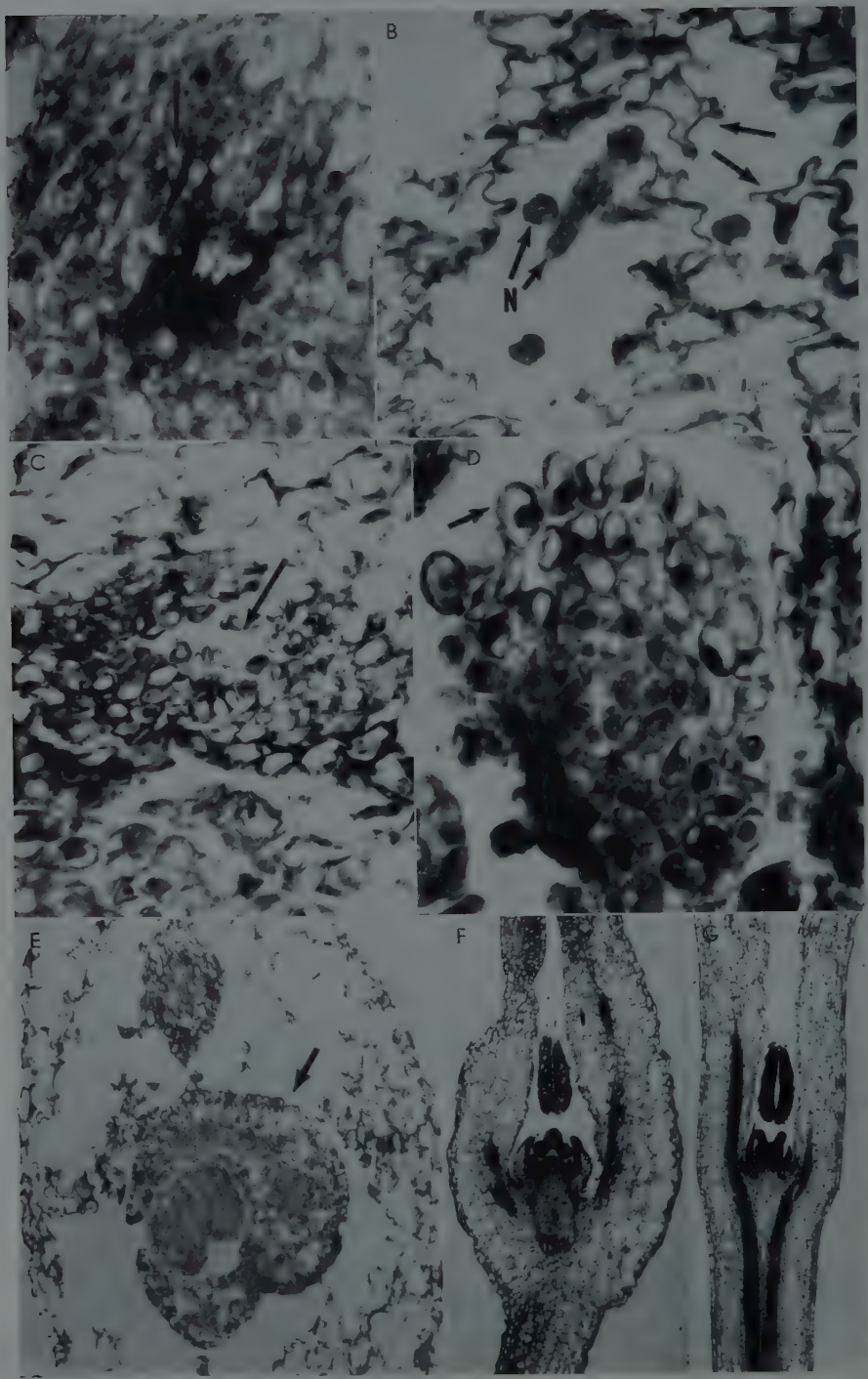
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6 hours after inoculation. At one point of attack epidermal cells of a cotyledon had enlarged causing the epidermis to separate from the underlying cortical cells. Ten to twelve cells in this area were discolored and distorted (Fig. 1, E). At other points where epidermal cells had been penetrated reactions in surrounding cells were visible several cells away from that point, but the epidermis was not buckled. Nematode damage at this stage sometimes appeared to be caused directly by penetration of cells rather than diffusion of substances into surrounding cells.

In one shoot nematodes penetrated directly downwards into a cotyledon base to a cotyledonary trace (Fig. 2, A). The bottom of the cavity was surrounded by amorphous, darkly-stained material and walls of nearby xylem elements had thickened and darkened.

18 hours — Damage to some tissues was more obvious than at 12 hours, but otherwise similar. Shoot apices and embryonic leaves were particularly affected. Epidermal cells of some apices were distorted by nematode attack and were highly vacuolated; in other apices epidermal and underlying cells were swollen and separating, but not vacuolated or distorted. Affected cells contained dense, granular, purple-stained cytoplasm, enlarged grayish nuclei and large bright red nucleoli. Cortical parenchyma or epidermal cells adjacent to nematode-penetrated or discolored areas were enlarged. At this time the beginnings of gall formation were visible.

24 hours — At 24 hours yet other cellular and tissue abnormalities were caused by the nematode. Some cortical parenchyma cells, projecting into cavities caused by nematodes in the cortex, were greatly misshapen (Fig. 2, B), although still apparently alive. Sometimes only the cell wall portions directly exposed to the cavity were misshapen while the remainder was normal. Probably these cells were originally isodiametric, but the walls had assumed these grotesque shapes without the cells being killed. All the cortical cells of a stem or petiole in the area of a cavity were often separated from one another sufficiently to allow nematodes to pass between them; however, nematodes still occurred only inside cavities.

Vascular tissues were also affected. Cells of vascular bundles were separated from one another as were cortical cells in infected stems (Fig. 2, C). Tracheary elements were separated from the vascular parenchyma which showed abnormalities similar to those seen in affected cortical cells, such as enlargement, granular cytoplasm, discoloration, and enlargement of nuclei and nucleoli. These effects

Fig. 2. Alfalfa seedling tissues infected by *D. dipsaci*. A.) Cross section of a stem about 1 mm below a cotyledon base 12 hours after nematode inoculation. Note the deeply-stained cells around the cavity and the stained walls of nearby xylem elements (arrow). × 409. B.) Cross section of an infected cotyledon petiole 24 hours after nematode inoculation. Note the nematodes (N), deformed parenchyma cells (arrows) and abundant intercellular space. × 391. C.) Cross section of a stem at a cotyledon base 24 hours after nematode inoculation. Note separation of cells in the vascular bundle (arrow). × 386. D.) Cross section through a shoot apex 24 hours after nematode inoculation. Note irregular profile of the epidermis (arrow) × 401. E.) Cross section of a shoot apex 36 hours after nematode inoculation. Note separation of cells of the embryonic leaf (arrow). × 93. F.) Longisection of a shoot apex 2 days after nematode inoculation. × 33. G.) Longisection of a healthy shoot apex of the same age as in F.

extended further, both acropetally and basipetally, in vascular tissue than in cortex, suggesting that the stimulus causing these reactions had been transported rather than had diffused.

Affected apex cells of shoots were often very distorted (Fig. 2, D). Epidermal cells had enlarged and protruded externally, distorting the cuticle and causing the epidermis to separate from the underlying cells. All of these apical cells were discoloured and displayed the typical abnormalities due to nematode damage. Some of the apical meristem cells were enlarged, vacuolated, and separated enough to permit the passage of nematodes between them although none were seen in this tissue.

36 hours — Nematodes had caused a great deal of damage in infected tissues and stem galling was easily visible without magnification. Many cortical cells were completely destroyed and the epidermis near the cotyledon base, at the point where infection had occurred, was missing (Fig. 2, E). Embryonic leaves were severely affected; cells were considerably enlarged and separated leaving many large intercellular spaces. Where infections extended into the cortex below the apical region, much of the cortex was destroyed although the epidermis and vascular tissues remained intact. In some areas of infected cotyledons, cells of the lower epidermis had enlarged causing the entire epidermis to buckle away from the mesophyll (Fig. 1, F). Although only very few nematodes were found, infected cotyledons had larger cross-sections than uninfected cotyledons and contained much more air space.

Later Stages of Parasitism of Alfalfa by D. dipsaci *and* A. ritzemabosi

D. dipsaci, *plants in soil* — Cortical parenchyma cells in infected cotyledon bases were considerably enlarged compared with cells in healthy shoots two days after nematode inocubation (Fig. 2, F, G). Alreary most of the chloroplasts were missing from gall parenchyma cells imparting a light green or whitish color to the galls. Nematodes frequently destroyed many of these enlarged cortical cells in forming cavities inside the galls (Fig. 3, A). By this time nematodes were moving freely between the nearby separated parenchyma cells on the edge of the galled area. As the galls increased in size, destruction of cortical cells and enlargement of epidermal cells continued. Nematodes initiating galls in leaf bases tended to move upward in the stem cortex, increasing the size of the gall acropetally, and also into the leaf petiole, but they also penetrated basipetally into the stem cortex, although to a lesser extent. Eggs were seen in gall cavities ten days after inoculation.

After 20 days infected stem tissues just above an internode appeared as seen in Fig. 3, D. The epidermis was expanded so that it became convoluted, much of the cortex was destroyed and the remaining cells were misshapen. Nematodes were seen in cavities in the pith. Pith was entered by nematodes penetrating through the parenchyma of the leaf gaps. Direct damage was restricted to parenchyma of the cortex and pith, with no separation of cells in vascular bundles as occurred 24 hours after inoculation (Fig. 2, C). Even after 20 days often only five or ten nematodes

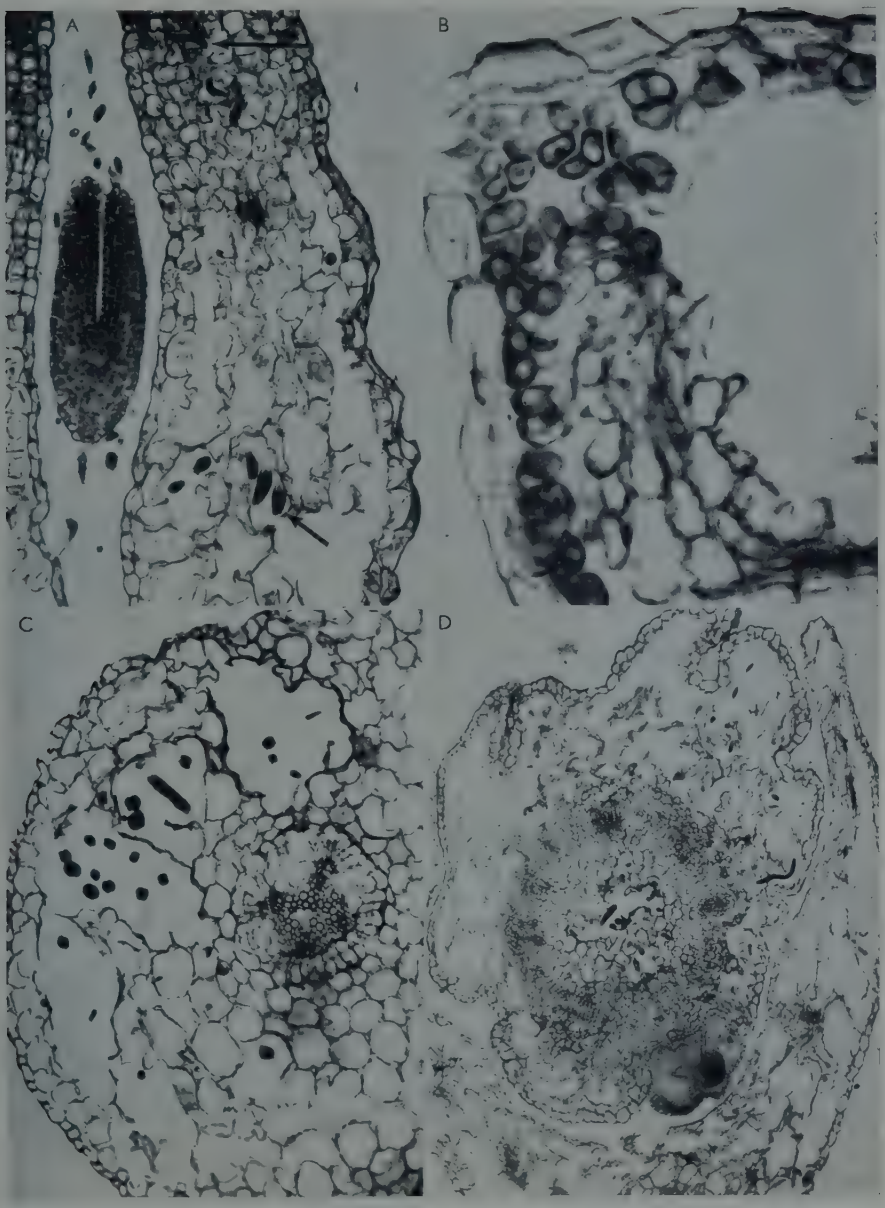

Fig. 3. Alfalfa tissues infected by *D. dipsaci*. A.) Longisection of a cotyledon petiole 2 days after nematode inoculation. Note nematodes (arrows) × 98. B.) Cross section of an infected leaf 20 days after nematode inoculation. Note the large cavity and rounded cells containing dense protoplasm. × 368. C.) Cross section of an infected root 20 days after nematode inoculation. × 89. D.) Cross section of an infected stem 20 days after nematode inoculation. × 31.

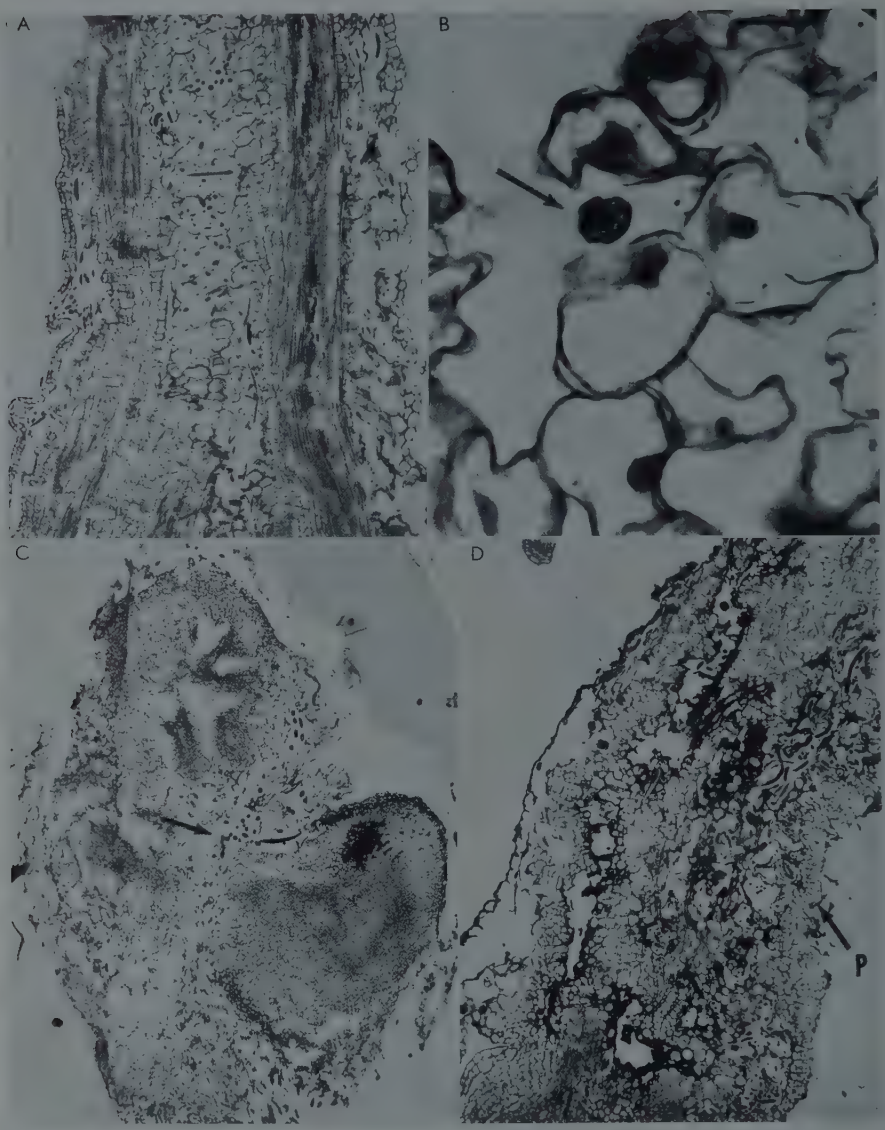

Fig. 4. *D. dipsaci* infecting alfalfa stems and callus tissue. A.) Longisection of an infected stem 45 days after nematode inoculation. × 30. B.) Cross section through the cortex of a galled stem 20 days after nematode inoculation. Nematode (arrow). × 386. C.) Section through callus tissue 30 days after nematode inoculation. Group of nematodes (arrow). × 30. D.) Longisection of an infected stem 60 days after nematode inoculation. Periderm (P.) × 30.

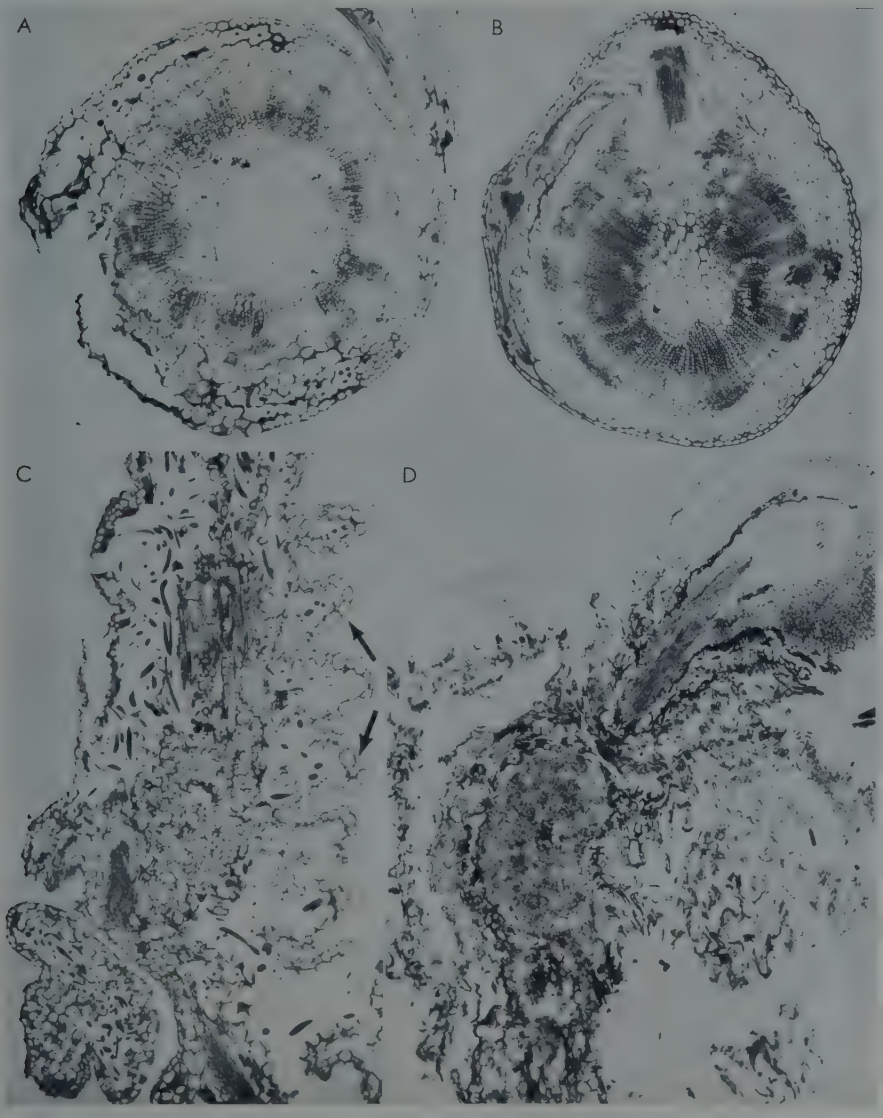

Fig. 5. Alfalfa stems infected by *D. dipsaci* and callus infected by *A. ritzemabosi.* A.) Cross section of a stem 45 days after inoculation with *D. dipsaci.* × 42. B.) Cross section of a healthy stem from a 60-day-old plant. × 42. C.) Longisection of an infected cotyledon petiole 45 days after inoculation with *D. dipsaci.* Note the convoluted epidermis (arrows). × 33. D.) Section of callus 45 days after inoculation with *A. ritzemabosi.* × 33.

Fig. 6. Alfalfa parasitized by *A. ritzemabosi.* A.) Longisection of the leaf axil region of a parasitized shoot 60 days after nematode inoculation. × 102. B.) Cross section of an infected cotyledon lamina 10 days after nematode inoculation. Nematode (arrow). × 102.

were found in extensive galls. Apical growth of young infected plants was frequently completely inhibited.

At 45 days after inoculation, epidermal cells around galls were greatly enlarged and often caused the epidermis to become convoluted (Fig. 5, C.). Gall cavities were full of nematodes and eggs. By this time nematodes had penetrated to and damaged some vascular strands. Pith was colonized extensively by nematodes and many eggs were found in the nematode cavities there (Fig. 4, A). Penetration by nematodes into uninfected cortex of old plants resulted mostly in destruction of the outer cortex beneath the epidermis with very little galling (Fig. 5, A). Intact parenchyma cells of this inner cortex remained less differentiated than in comparable healthy stems (Fig. 5, B). These cortical cells were small, densely protoplasmic and frequently contained mitotic figures. Vascular parenchyma cells in stem galls occasionally contained mitotic figures. Many possessed large nuclei and nucleoli. After 60 days the epidermis and most of the outer cortex had sloughed off in heavily infected stems (Fig. 4, D), and a periderm layer formed in the remaining cortex. Cavities in such stems contained many nematodes. Cavities formed by nematodes were usually lined with much red-staining material. Stem infections on old plants, where little galling occurred, caused little inhibition of shoot growth.

Leaf galls were formed within ten days after nematode inoculation. Despite the presence of only one or two nematodes in galls most of the spongy mesophyll and part of the palisade mesophyll had been destroyed leaving a large amount of air space (Fig. 3, B). The lower epidermis was greatly expanded. Chloroplasts in the remaining palisade cells appeared to have dissolved to form a layer together with the cytoplasm of dense gray-staining granular material inside the cell wall. These cells were slightly enlarged and rounded and each contained a large central vacuole. Nuclei were enlarged and granular, and contained large bright red nucleoli. A few cells were dividing mitotically. Some cortical parenchyma cells of stem galls were similar in appearance (Fig. 4, B).

Various cellular distortions were seen in galled tissues of all ages. Epidermal and cortical cells were sometimes, but not always, ten or more times larger than those present in uninfected parts of the same plant, or in comparable tissues of healthy plants. In addition to separating from one another, leaving large intercellular spaces, these intact cortical cells in galled tissue usually had misshapen cell walls, and both the nuclei and nucleoli were enlarged five to ten times and often vacuolated (Fig. 4, B). Mitotic figures were occasionally observed in these cells. Cells sometimes contained two to five nuclei which were frequently elongate, pear-shaped, barbell-shaped or otherwise distorted, and often contained several enlarged and distorted nucleoli.

Nematodes sometimes fed ectoparasitically on embryonic tissues of axillary buds or on shoot apices and were often present between these tightly rolled parts. Epidermal layers of embryonic tissues were frequently necrotic due to nematode feeding and failed to expand, or were distorted when expanded.

Galls from naturally infected plants in the field were identical histopathologically to those formed under greenhouse conditions.

D. dipsaci, *sterile plants* — Galls caused by *D. dipsaci* on shoots of sterile alfalfa seedlings growing on nutrient agar medium were identical histopathologically with galls produced on plants growing in soil. Tissue and cell abnormalities were similar in both cases and a like amount of necrosis occurred in galls formed in the absence of other micro-organisms.

Nematodes were often found inside alfalfa roots growing through the agar medium (Fig. 3, C). Five days after inoculation the first nematodes were found inside small cavities in roots and by 30 days large cavities containing many nematodes and eggs had been formed. In roots, nematodes were observed only in cavities in the cortex. Apparently nematodes entered roots through the ruptured tissues where lateral roots had emerged from the parent root. Cavity formation was the main distortion in infected roots. Even after 60 days there was very little necrotic tissue around cavities. Parenchyma cells surrounding cavities were little affected by the presence of nematodes; cells did not enlarge, divide or separate and the cytoplasm of cells bordering cavities was unchanged although their nuclei were enlarged and had large bright red nucleoli.

D. dipsaci, *callus tissue* — A loose mass of callus parenchyma was produced from rapidly dividing pericycle and cortical parenchyma derived from the roots of sterile alfalfa seedlings growing on the nutrient agar medium containing 2,4-D, the shoot having atrophied after a few weeks. The parenchyma cells were larger than normal and generally very misshapen. Isolated or small groups of parenchyma cells in the center of a mass sometimes differentiated into tracheary elements. Many parenchyma cells had enlarged nuclei and nucleoli; the cytoplasm was often gray-colored and dense, and some cells were binucleate.

Pustule-like growths occurred at the apices of main and lateral roots even when lateral roots had not yet pushed through the cortex of the parent root. Centers of these pustules were frequently filled with masses of tracheary elements. Surrounding the xylem was a thick layer of meristematic cells, apparently derived from the cortex, pericycle and vascular cambium. These divided to form the large external mass of parenchyma and more xylem.

Nematodes were usually scattered through the loose parenchyma of the callus, but occasionally occurred in masses in cavities. A few eggs were found in the callus five days after nematode inoculation and frequently thereafter. Nematodes had very little effect on the callus except where they destroyed cells during cavity formation. Occasional cells were visibly affected by nearby nematodes; these had large granular nuclei and bright red nucleoli, dense granular cytoplasm and slightly thickened cell walls. Nematodes occasionally penetrated to vascular tissues in callus and were found in cavities beside tracheary elements. None penetrated the dense meristematic cell mass of pustules. Old infected callus contained some areas of necrotic cells, but these were never extensive enough to kill the tissue cultures. Later, when many nematodes were present, the loose callus parenchyma often partially disintegrated and appeared in a watery layer over the agar surface. Callus, 60 days after infection, was much darker brown than nematode-free callus, and

most of the nematodes were in the agar medium. Apparently nematodes leave the callus and return to feed at intervals. Fig. 4, C shows callus tissue containing nematodes. The loose callus parenchyma resembled the distorted parenchyma of galled stem cortex (Fig. 3, D).

A. ritzemabosi, *plants in soil — A. ritzemabosi* gathered in young tightly-rolled buds of alfalfa plants and fed almost entirely ectoparasitically on the epidermal cells of embryonic leaves and the shoot apex. Feeding killed the outer 2-3 layers of cells and caused a thick red-stained layer to form over these organs (Fig. 6, A). The red-stained layer inhibited growth and division of the underlying intact cells which became enlarged, vacuolate, contained little cytoplasm, and whose nuclei were large, reddish and granular with apparently normal nucleoli. Growth of the entire shoot was inhibited. When about five gm of stunted shoots were placed in Baermann funnels overnight thousands of nematodes were recovered.

Of the few nematodes that penetrated parts of the shoot some were in the lamellae of alfalfa cotyledons ten days after inoculation (Fig. 6, B). They apparently penetrated through stomata, because specimens were often seen in substomatal cavities and the accompanying guard cells were swollen, causing the apertures to enlarge. Cavities in which many eggs were seen were most frequent in the spongy mesophyll especially around vascular bundles; the palisade mesophyll was entered only occasionally; infections were usually accompanied by necrosis. Few chloroplasts remained in cells around infections and frequently the cells contained large distorted nuclei with large bright red nucleoli. A few nematodes were seen in necrotic stem tissues, but never in living tissues.

A. ritzemabosi, *sterile plants* — The damage done here was similar to that done to plants in soil. Most of the nematodes seemed to feed on roots growing through the agar medium rather than on shoots. Clusters of nematodes were observed around root apices, and numerous eggs were present there and scattered further back along roots.

A. ritzemabosi, *callus tissue* — Nematodes were generally scattered through the loose callus mass and only occasionally occurred in groups — the case with *D. dipsaci.* Eggs were very numerous. Nematodes were frequent around the meristematic cells of pustules and penetrated these dense cell masses causing some adjacent intact cells to become necrotic and others to become enlarged and vacuolated. Specimens sometimes penetrated to the xylem. Often a layer of red-staining substance covered the dense central part of the pustule (Fig. 5, D), similar to that in shoot apices of infested seedlings (Fig. 6, A). Sometimes cavities lined with the red-stained substance were formed in this dense tissue.

In older cultures *A. ritzemabosi,* which reproduced more rapidly than *D. dipsaci,* caused more rapid, although similar, disintegration of callus masses, and old infected callus cultures were darker brown, with only the central region intact. Although enlargement of some pustules was prevented by nematode parasitism, a few pustules in any one culture were still enlarging 60 days after nematode inoculation.

Biochemical

Free glucose, fructose and sucrose were detected in all samples of plant tissues regardless of whether or not *D. dipsaci* or *A. ritzemabosi* were present or under what conditions the tissues were grown. Assays of massed nematodes showed glucose and fructose in both species.

Free organic acids were also similar in healthy and nematode-parasitized plant tissues grown under all three conditions. Acids identified were oxalic, tartaric, citric, malic, malonic and succinic. Glycolic acid was found in plants grown in soil, but not in sterile plants or callus tissue grown on agar media. No organic acids were identified from nematode extracts although some were present.

The conditions under which the plants or tissues were grown, and the presence or absence of nematodes had no effect on most of the principal amino acids of the many present in the extracts of the various alfalfa tissues. Free amino acids identified in all extracts were: aspartic acid, glutamic acid, serine, glycine, threonine, alanine, proline, histidine, arginine, asparagine, methionine, valine, glutamine, leucine-isoleucine, phenylalanine, gamma-aminobutyric acid and cysteic acid. Thirteen other ninhydrin-positive compounds produced distinct spots on the various chromatograms. Most of these occurred inconsistently in small spots and no attempts were made to identify them

The occurrence of free tryptophane was interesting. It was found only in plant galls caused by *D. dipsaci,* and in all callus tissue regardless of the presence or absence of either species of nematodes. Healthy plants or plants parasitized by *A. ritzemabosi* growing in soil or under sterile conditions on agar medium contained no free tryptophane.

Free tyrosine was present in extracts of plants or callus parasitized by *A. ritzemabosi,* but absent, or present only in traces, in plant tissues free from nematodes or parasitized by *D. dipsaci.* All hydrolysates contained abundant tyrosine.

Most of the amino acids present in hydrolysates were the same as those found free in plant tissues. Amino acids found free, but not in hydrolysates, were asparagine, tryptophane, glutamic and gamma-aminobutyric acid. Those present in hydrolysates, but not free, were hydroxyproline, lysine and alpha-aminobutyric acid.

Specimens of *D. dipsaci* and *A. ritzemabosi* were enough for only one extract of each species. Free amino acids identified from the *D. dipsaci* extract were aspartic acid, glutamic acid, serine, glycine, threonine, alanine, tyrosine, histidine, arginine, valine, leucine-isoleucine, gamma-aminobutyric acid and cysteic acid. Extracts of *A. ritzemabosi* contained aspartic acid, glutamic acid, serine, glycine, threonine, alanine, arginine, valine, gamma-aminobutyric acid, cysteic acid, lysine and methionine. gamma-Aminobutyric acid was absent in the hydrolysates, but in addition to all the other amino acids mentioned above, proline, hydroxyproline and phenylalanine were found. The chromatogram of the hydrolysate of *A. ritzemabosi* contained two spots and that of *D. dipsaci* one of the same spots which

did not occur on any of the chromatograms of plant extracts. No attempts were made to identify these.

Extracts of the agar media supporting growth of alfalfa seedlings and callus still contained too many interfering substances, after passage through ion exchange resin columns, for sharp separation of amino acids on paper chromatograms. Quantities of amino acids in the agar media were also low. The occurrence of amino acids in these extracts was inconsistent from one replication to another, though seventeen amino acids were identified from the various extracts.

DISCUSSION

The difficulty in obtaining nematodes in quantity has greatly handicapped many types of studies with these plant parasites. Some species can now be obtained in gram quantities with relative ease using monoxenic cultures on alfalfa callus tissue. This technique places research with plant nematodes on a basis similar to that with many phytopathogenic fungi. The uses to which nematode cultures can be put are many, e.g., maintenance of pure nematode populations; studies of nematode biology, genetics and nutrition; and propagation of masses of nematodes needed to study nematode physiology and composition, and for screening nematicidal compounds. Some species of nematodes have reproduced only slowly or not at all on alfalfa callus. Further studies will be needed with various plant tissues and media before all plant-parasitic nematodes are cultured in this manner.

A useful feature of monoxenic plant callus-nematode cultures is that they can be stored for at least two years without subculture and still contain many live nematodes. The aluminum foil caps sealed to the test tubes with cellophane tape prevent the agar medium from drying, but allow enough gas exchange for the plant tissues and nematodes to survive.

Specimens of *P. zeae* reproduced on alfalfa callus tissue, but not on seedlings. By determining how cultures of plant tissues are altered biochemically and physiologically under the influence of various media information on the mechanisms of resistance of plants to nematodes might be gained.

The histopathology of *D. dipsaci* on alfalfa plants was in many ways similar to the effects of this nematode on potato plants as described by Quanjer (1927). Nematodes entered through natural openings on potato leaves, stems and tubers, e.g., stomata and lenticels, whereas they penetrated directly through the epidermis in leaf bases on alfalfa seedlings. Cell enlargement, disappearance of chloroplasts and increase in intercellular space in parenchyma tissue were similar in both potato and alfalfa. Effects often preceded direct contact of nematodes with the cells, suggesting that salivary secretions diffused in advance of the nematodes. In older infections cork appeared in both potato tubers and alfalfa, and seemed to result from the presence of necrotic tissues rather than directly from nematode influence. Cork did not seem to restrict nematode activity in plant tissues.

Cells with dense granular protoplasm were formed from palisade parenchyma of alfalfa leaves infected by *D. dipsaci* (Fig. 3, B). These cells appeared to be

meristematic and a few contained mitotic figures. Similar cells were seen in infected cortical parenchyma of stems (Fig. 4, B) and affected embryonic tissues (Fig. 2, D). J. B. Goodey (1939) called such cells in *D. dipsaci*-galled leaves of *Plantago lanceolata* L. "nutritive" cells. He suggested that cells assumed this appearance following accumulation of a high concentration of salivary secretions precipitating substances in the protoplasm. Entire leaflets of alfalfa were galled and masses of such cells occurred with only one or two nematodes in a leaf. In galled alfalfa stems only localized cells in the parenchyma mass were dense and granular. Thus the younger or more meristematic cells seem to be highly sensitive to the presence of nematodes, as T. Goodey (1935) observed.

The histopathology of galls caused by *D. dipsaci* on alfalfa plants growing in soil and those growing aseptically on nutrient agar medium were identical. This indicates that other microorganisms have little influence on the galling of alfalfa.

The reactions of plant tissues to infection by *D. dipsaci* have often been discussed (Quanjer, 1927; T. Goodey, 1929, 1935; J. B. Goodey, 1939). Seinhorst (1959) states that plants react in two ways to this nematode, 1) dissolution of the middle lamellae between parenchyma cells which is essential to nematode feeding and reproduction, and 2) swelling and distortion of tissues which is non-essential to the nematode.

Pectinases have long been considered to be involved in the parasitism of plants by *D. dipsaci.* Tracey (1958) and Krusberg (1960) could not detect pectinases in homogenates of massed specimens of *D. dipsaci,* but this does not prove that pectinolytic enzymes are lacking in this nematode. Pectinases may not be involved in parasitism of alfalfa by this nematode. According to Wood (1960) plant cells die rapidly after maceration by pectinases, but cells do not seem to die in nematode-galled plant tissues. Ruthenium red is not a specific stain for pectins (Wood, 1960), so the observations by T. Goodey (1929) that the middle lamellae of parenchyma cells in nematode-infected potato tubers and narcissus bulbs were much less densely stained by ruthenium red than in healthy tissue does not prove a quantitative difference in pectins. In the culture experiments reported here, nematodes broke down agar, which is composed mainly of long chains of galactose molecules hooked together in 1 : 3 linkages (Bonner, 1950), but the extent of hydrolysis was not measured. Pectin is composed of long chains of galacturonic acid molecules hooked together by 1 : 4 linkages. The role of these enzyme systems in the disease is being further studied.

J. B. Goodey (1948) suggested that plant growth hormones were involved in galls formed by plant parasitic nematodes, and evidence favors this view. In the work reported here and that by T. Goodey (1935), very young, actively growing plant tissues reacted most to nematode infection and also reacted most to plant growth hormones. Old tissues which became infected, or cortex, where infections extended basipetally into the stem below the apical region, were only slightly or not at all galled and surrounding cells in the infected area were not separated (Fig. 5, A) as would happen if pectinases were present. Infected roots

were not galled. The similarity in structure of nematode-gall tissue (Fig. 3, D) and callus tissue induced by 2,4-D (Fig. 4, C) also suggests growth hormone activity in galls. Nolte (1952) found that extracts from plants infected with *D. dipsaci* contained more auxin or some plant growth substance than healthy plants.

The finding of free tryptophane in alfalfa galled by *D. dipsaci,* but not in healthy plants is further evidence that indoleacetic acid (IAA) might be involved in the galling reaction. Some of this free tryptophane could easily be converted to IAA by enzymes in the nematode or plant and if this occurred IAA could be the actual cause of galling. Callus tissue also contained free tryptophane which indicates that 2,4-D somehow caused the accumulation of this amino acid. In plants the nematodes might actually secrete IAA, or cause the plant to accumulate IAA in the vicinity of the nematodes. The tryptophane might be formed from an excess of auxin. Enlargement, distortion and pulling apart of cells was seen 12 hours after inoculation of alfalfa plants with *D. dipsaci* which suggests that nematodes contain plant growth substances. Nolte & Köhler (1952) reported that extracts of this nematode had growth promoting activity on sunflower seedlings. Plant growth substances would likely be more important than pectinolytic enzymes in the galling reaction of plants to nematodes.

A. ritzemabosi fed primarily as an ectoparasite on alfalfa plant shoots and roots of sterile plants growing on agar medium. However, nematodes were found in the laminae of some cotyledons and necrotic tissues of stems and stipules. In the greenhouse alfalfa seedlings were severely stunted and deformed by this nematode, and this type of parasitism is typical for this nematode, but such damage has not been observed in the field.

Alfalfa callus tissue was an excellent substrate for reproduction of both *D. dipsaci* and *A. ritzemabosi* which feed in parenchyma and callus provides a ready-made substrate composed of an abundance of such cells. The relationship of nematodes to callus is balanced and the plant tissues are damaged very little, as might be expected with obligate parasites. Only when large nematode populations developed were the plant tissues damaged, but even then they were not killed.

Qualitatively, there were no differences in free sugars or organic acids and differences in only two major amino acids in the various healthy and nematode-parasitized alfalfa tissues. Qualitative assays of the substances might provide further information on biochemical alterations occurring in diseased tissues and help us understand the physiological relationships between plants and pathogens and how pathogens harm plants. Owens & Novotny (1960) found that tomato and cucumber plant roots galled by a root-knot nematode (*Meloidogyne incognita*) contained four times as much free amino acids and amides as healthy roots.

Free tyrosine was abundant in plants and callus parasitized by *A. ritzemabosi,* but was present only in traces or lacking in healthy tissues or those infected by *D. dipsaci.* Leaves of chrysanthemum and several other plants rapidly turn brown when infected by *A. ritzemabosi,* and heavily infected alfalfa callus was darker brown than nematode-free callus. Polyphenol oxidase acting on free tyrosine might

account at least in part for the discoloration of plant tissues infected by this nematode.

The two unidentified amino acids in the acid hydrolysates of nematode specimens deserve further study. They appeared to be components of more complex molecules, and analogs of these might be prepared which would act as specific antimetabolites towards the nematodes, i.e., as specific nematicides.

The free amino acids found in plants were not qualitatively the same as those present in the acid hydrolysates of the alcohol-extracted residues. If nematodes do secrete proteolytic enzymes into plants, the plant proteins are not completely hydrolyzed. Some amino acids, e.g., tryptophane, are destroyed by acid hydrolysis. The significance of these differences is not known.

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ZUSAMMENFASSUNG

Untersuchungen über die Kultivierung und den Parasitismus pflanzenparasitärer Nematoden besonders Ditylenchus dipsaci *und* Aphelenchoides ritzemabosi, *an Luzernegewebe*

Ditylenchus dipsaci und *Aphelenchoides ritzemabosi* vermehrten sich lebhaft auf Luzernekallusgewebe, welches auf einem Nährагarmedium mit Zusatz von 2,4-Dichlorphenoxyessigsäure (2,4-D) wuchs. Die mit je 50 Nematoden inokulierten und bei einer Temperatur von 22° C gehaltenen Kulturen enthielten nach 2 Monaten 40,000 bis 80,000 Nematoden. Sterile Sämlinge, welche auf dem gleichen Medium, jedoch ohne Zusatz von 2,4-D wuchsen, ergaben ungefähr 25% der genannten Nematodenmenge. *Pratylenchus zeae* und *P. penetrans* vermehrten sich gut, *Tylenchorhynchus capitatus* und *Hoplolaimus coronatus* vermehrten sich langsam auf Luzernekallus. *D. dipsaci, P. zeae, T. capitatus* und *H. coronatus* wurden in solchen Kulturen 2 Jahre lang ohne weitere Übertragung auf neue Kulturen am Leben gehalten.

Die Histopathologie von *D. dipsaci* und *A. ritzemabosi* wurde untersucht unter Verwendung von in Erde gezogenen Luzernensämlingen, aseptisch auf Nährагarmedium gezogenen Sämlingen und aseptisch auf Nährагarmedium mit 2,4-D wachsendem Kallusgewebe. Versuche mit *D. dipsaci* an Luzernesämlingen in Erde zeigten, dass diese Art direkt durch die Epidermis am Grunde der Keimblätter eindrang, Höhlungen im Rindenparenchym bildete und die Spitzenregion innerhalb 12 Stunden nach der Beimpfung erreichte. Allgemeine Schwellung der infizierten Stengel konnte frühestens 36 Stunden nach der Beimpfung mit Nematoden beobachtet werden, obwohl kleine Zellgruppen schon vorher anschwollen und sich voneinander trennten. Die Infektion zerstörte viele Parenchymzellen. Andere zeigten verzerrte Zellwände, granuliertes und vakuolisiertes Zellplasma, vergrösserte Nuklei und Nukleoli und in einigen Zellen kamen mitotische Kernteilungen vor. Junge, lebhaft wachsende Gewebe zeigten am meisten Gallbildung und Zelltrennung. Die Wurzeln der aseptisch auf Agarmedium wachsenden Sämlinge wurden infiziert und es bildeten sich grosse Höhlungen in der Rinde, jedoch erfolgte keine Gallbildung oder Zelltrennung. Die an Sprossen der in Erde wachsenden Luzernepflanzen erzeugten Gallen erwiesen sich histopathologisch identisch mit den an Sprossen der aseptisch auf Agarmedium wachsenden Pflanzen vorkommenden Gallen. *A. ritzemabosi* ernährte sich hauptsächlich als Ektoparasit an den Sprossen der in Erde oder auf Agarmedium wachsenden Luzernesämlingen und verhinderte Spitzenwachstum und Entwicklung der embryonalen Gewebe. Das Ergebnis waren schwer im Wachstum zurückgebliebene und missgestaltete Pflanzen. Einige Nematoden drangen in die Keimblattspreiten ein und lebten und vermehrten sich in deren

Schwammparenchym, ohne viel Schaden anzurichten. Sowohl *D. dipsaci* als auch *A. ritzemabosi* ernährten und vermehrten sich im äusseren lockeren Parenchym des Luzernekallus und verursachten geringen Schaden, ausser wenn viele Nematoden vorhanden waren. In alten Kulturen hatten sich die meisten Nematoden vom Kallusgewebe auf das Agarmedium und die Wände der Kulturgefässe begeben.

Die Luzernegewebe wurden auf das Vorkommen von Zucker, organischen Säuren und Aminosäuren geprüft. Weder die Nematoden noch die Bedingungen, unter welchen die Pflanzen oder Gewebe gezüchtet wurden, hatten einen qualitativen Einfluss auf die vorhandenen Zucker und organischen Säuren; nur 2 der 23 wichtigsten identifizierten Aminosäuren wurden beeinflusst. Gallengewebe der mit *D. dipsaci* infizierten Pflanzen und alle Kallusgewebeproben enthielten freies Tryptophan, während in gesunden oder von *A. ritzemabosi* parasitierten Pflanzen kein Tryptophan nachgewiesen werden konnte. Die von *A. ritzemabosi* befallenen Gewebe enthielten reichlich freies Tyrosin, während nematodenfreie oder mit *D. dipsaci* infizierte Gewebe wenig oder kein Tyrosin enthielten.

LITERATURE CITED

Block, R. J., Durrum, E. L. & Zweig, G. (1958). *A manual of paper chromatography and paper electrophoresis.* Academic Press, New York, 2nd edition, 710 pp.

Bonner, J. (1950). *Plant Biochemistry.* Academic Press, New York, 537 pp.

Bryant, F. & Overell, B. T. (1953). Quantitative chromatographic analysis of organic acids in plant tissue extracts. *Biochim. biophys. Acta* **10**, 471-476.

Buch, M. L., Montgomery, R. & Porter, W. L. (1952). Identification of organic acids on paper chromatograms. *Analyt. Chem.* **24**, 489-491.

Darling, H. M., Faulkner, L. R. & Wallendal, P. (1957). Culturing the potato rot nematode. *Phytopathology* **47**, 7. (Abstr.)

Dropkin, V. H. & King, R. C. (1956). Studies on plant parasitic nematodes homogeneously labeled with radiophosphorus. *Exp. Parasitol.* **5**, 469-480.

Endo, B. Y. (1959). Responses of root-lesion nematodes, *Pratylenchus brachyurus* and *P. zeae,* to various plants and soil types. *Phytopathology* **49**, 417-421.

Goodey, J. B. (1939). The structure of the leaf galls of *Plantago lanceolata* L. induced by *Anguillulina dipsaci* (Kühn) Gerv. and v. Ben. *J. Helm.* **17**, 183-190.

—— (1948). The galls caused by *Anguillulina balsomophila* (Thorne) Goodey on the leaves of *Wyethia amplexicaulis* Nutt and *Balsomorhiza sagittata* Nutt. *J. Helm.* **22**, 109-116.

—— (1957). *Laboratory methods for work with plant and soil nematodes.* Tech. Bull. 2, Minist. Agric., London, H.M.S.O. 3rd edition, 47 pp.

Goodey, T. (1929). The stem eelworm, *Tylenchus dipsaci* (Kühn, 1958): Observations on its attacks on potatoes and mangolds with a host list of plants parasitized by it. *J. Helm.* **7**, 183-200.

—— (1935). *The pathology and aetiology of plant lesions caused by parasitic nematodes.* London, Imp. Bur. Agric. Parasitol. Pub. 34 pp.

Hildebrandt, A. C., Riker, A. J. & Duggar, B. M. (1946). The influence of the composition of the medium on growth *in vitro* of excised tobacco and sunflower tissue cultures. *Amer. J. Bot.* **53**, 591-597.

Krusberg, L. R. (1960). Hydrolytic and respiratory enzymes of species of *Ditylenchus* and *Pratylenchus. Phytopathology* **50**, 9-22.

Mountain, W. B. (1955). A method of culturing plant parasitic nematodes under sterile conditions. *Proc. helm. Soc. Wash.* **22**, 49-52.

Nolte, H. (1952). Untersuchungen über die stofflichen Grundlagen bei Nematodenschaden *Mitt. biol. ZentAnst. Berl.* **74**, 40-42.

Nolte, H. & Köhler, H. (1952). Pflanzenschädigungen bei Nematodenbefall und ihre kausalen Ursachen. *NachrBl. Dtsch. Pflzschutzd. Berlin* **6**, 24-28.

Oteifa, B. A. (1952). Potassium nutrition of the host in relation to infection by a root-knot nematode *Meloidogyne incognita. Proc. helm. Soc. Wash.* **19**, 99-104.

—— (1953). Development of the root-knot nematode, *Meloidogyne incognita,* as affected by potassium nutrition of the host. *Phytopathology* **43**, 171-174.

Owens, R. G. & Novotny, Helen M. (1960). Physiological and biochemical studies on nematode galls. *Phytopathology* **50**, 650. (Abstr.)

PEACOCK, F. C. (1959). The development of a technique for studying the host/parasite relationship of the root-knot nematode *Meloidogyne incognita* under controlled conditions. *Nematologica* **4**, 43-55.

QUANJER, H. M. (1927). Een aaltjesziekte van de aardappelplant, de aantastingswijze en de herkomst van haar oorzaak, *Tylenchus dipsaci* Kühn. *Tijdschr. PlZiekt.* **33**, 137-172.

SASS, J. E. (1958). *Botanical microtechnique.* Iowa State College Press, Ames, 3rd edition, 228 p.

SEINHORST, J. W. (1959). The genus *Ditylenchus* and related genera. In: *Plant Nematology.* Edited by J. F. Southey. Minist. Agric. Tech. Bull. 7, London, H.R.S.O. pp. 33-43.

THOMPSON, J. F., MORRIS, C. J. & GERING, Rose K. (1959). Purification of plant amino acids for paper chromatography. *Analyt. Chem.* **31**, 1028-1031.

TRACEY, M. V. (1958). Cellulase and chitinase in plant nematodes. *Nematologica* **3**, 179-183.

TYLER, Jocelyn (1953). Reproduction without males in aseptic root cultures of the root-knot nematode. *Hilgardia* **7**, 373-388.

WIDDOWSON, Elizabeth, DONCASTER, C. C. & FENWICK, D. W. (1958). Observations on the development of *Heterodera rostochiensis* Woll. in sterile root cultures. *Nematologica* **3**, 308-314.

WOOD, R. K. S. (1960). Pectic and cellulolytic enzymes in plant diseases. *Annu. Rev. Pl. Physiol.* **11**, 299-322.

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A ROTATIONAL EXPERIMENT ON LAND INFESTED WITH POTATO ROOT EELWORM, *HETERODERA ROSTOCHIENSIS* WOLL.

BY

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The changes in potato root eelworm population under several different rotations were studied in a replicated experiment, in which potato yields were also obtained. Regressions of log final eelworm population on yield and of yield on log initial eelworm population were calculated, but yields were low probably for reasons other than eelworm.

This paper reports the results of a replicated trial, designed to study the changes in the potato root eelworm population (*Heterodera rostochiensis* Woll.) under three rotations and the relationship between eelworm population and yield.

The experiment was carried out from 1951 to 1953 on a loamy peat soil at Yaxley, Peterborough. Potatoes had failed in the previous year and, before the experiment, the average soil population was 43 eggs/g. The field (1.7 hectares) was divided into 12 plots each 61 m × 16.4 m giving three randomised blocks of 4 treatments.

The treatments were: —

I Potatoes every year.
II Potatoes in 1951 and 1953.
III Potatoes in 1952.
IV No potatoes.

Majestic was the variety of potato grown. Between the potato crops of treatments II and III and on the plots of treatment IV the following non-host crops were grown: — sugar beet, mixed corn (peas, beans, oats), winter wheat and carrots. Owing to poor seed, potato growth in the first year was indifferent and yields low. For this reason first-year yields were discarded. Weeds became troublesome at the end of the third year.

Every year the plots were sampled during the winter and the soil population of potato root eelworm estimated by the usual technique (Goodey 1957). Potato yields were estimated by lifting the two central rows of each plot (122 m of row). In July counts of volunteer potato plants were attempted on non-potato plots in five sample areas of 4.2 sq. m taken at random.

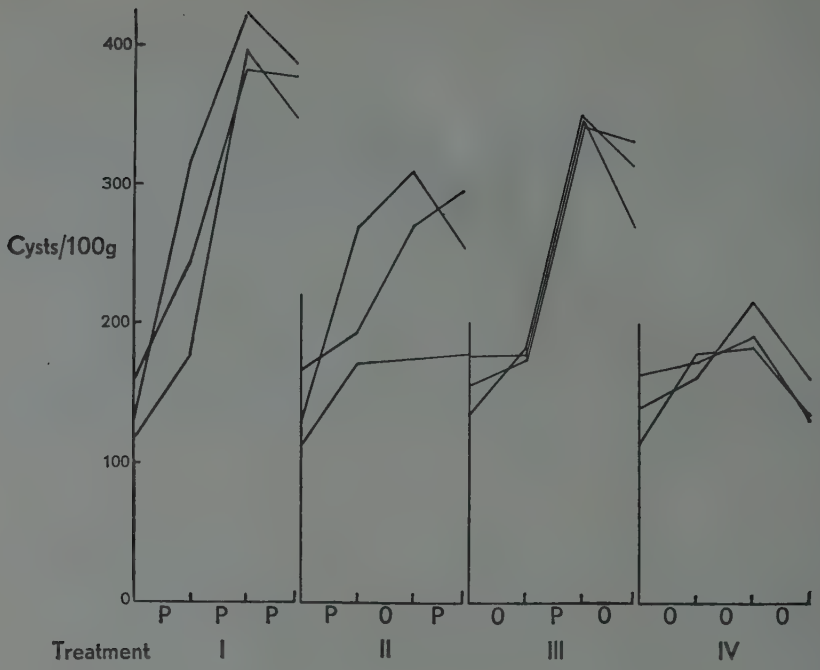

Fig. 1. Changes in total cyst populations with cropping (P = potatoes, O = non-host).

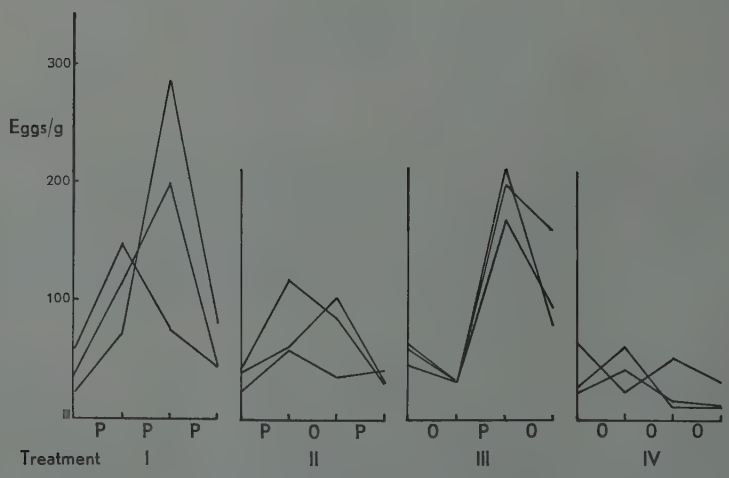

Fig. 2. Changes in egg populations with cropping (P = potatoes, O = non-host).

RESULTS

Population changes and yield

The changes in total cyst and egg populations for the 3 years on each of the twelve plots are shown in Figs. 1 & 2. They indicate that the eelworm population over the trial area was reasonably uniform initially and except perhaps for treatment II show fairly consistent population trends within each treatment. The results from the non-potato plots (Treatment IV) were unexpected. The total cyst counts on these plots rose in the first two years and, in the third year, were still slightly higher than at the beginning, although the egg counts on average fell gradually. It is unlikely that sampling errors can account for this rise as the three replicates behaved consistently and it is worth considering the possible reasons for it. The rise was not due to the movement of soil horizontally from adjacent plots as each plot was 16.4 m wide and soil sampling was restricted to the central 11 m. Moreover care was taken to cultivate only in the direction of the plots. Immediately after a crop has been lifted most of the newly formed cysts are in the top layer of the soil and after deep ploughing many of these are probably buried below the depth of normal sampling. This may possibly explain the apparently anomalous results especially those in treatments II and IV. Ideally sampling should be carried out to the actual depth of ploughing.

Changes in egg populations after potatoes show that at the lower initial levels the population has usually risen and at the higher levels it has usually fallen. At high initial population levels the larvae probably invade the roots in numbers greater than the roots can withstand and consequently both roots and larvae die. This may be a major factor accounting for the decrease in eelworm numbers under these conditions.

It has been shown by earlier workers (Chitwood & Feldmesser 1948, Oostenbrink 1950, Goffart 1952, Fenwick & Reid 1953, Schmidt 1954, Mai 1954 and Hague & Hesling 1958) that as the initial population rises so the multiplication rate falls. For example Chitwood & Feldmesser obtained increase rates for viable cysts varying from 7.54 to 0.69 and Mai quotes similar figures of 7.2 and 0.13. Hague & Hesling illustrate multiplication rates for eggs per gram varying from 50 for low initial populations to about unity for high initial populations. From data given by Oostenbrink increase rates for eggs per gram varying from about 150 to 0.01 can be calculated. In the present work (Fig. 2) the greatest multiplication was 6.6 with an initial population of 32 eggs/g and the lowest was 0.28 with 284 eggs/g.

The regression of yield on log initial egg population is shown in Fig. 3 for the second and third years. The correlation coefficient (r) for the second years was —0.739 (significant at $P = 0.01$) and for the third —0.402 (not significant).

The regression of log final egg population on yield is shown in Fig. 4, the correlation coefficient being 0.649 (significant at $P = 0.001$). This indicates that the final eelworm population tended to be higher on plots which yielded more heavily, as might be expected.

Ignoring the plots which yielded badly in the first year, the yields fell rapidly

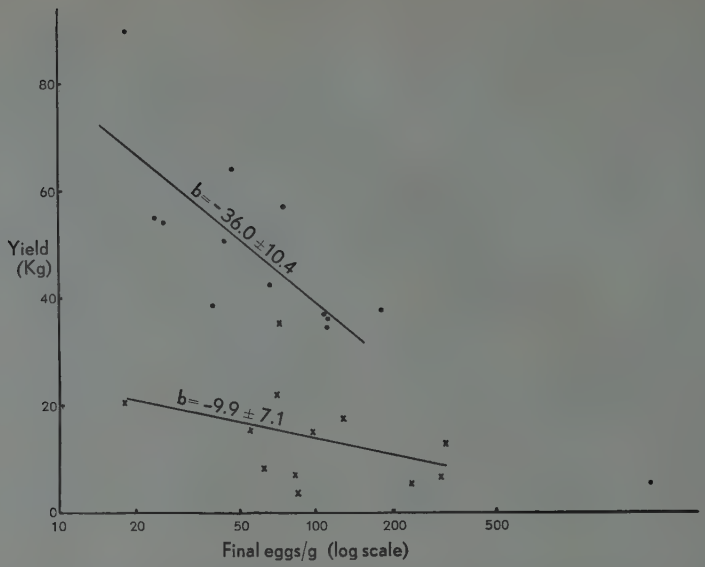

Fig. 3. The regressions of yield on log initial egg population. (10 kg ≡ 2200 kg per hectare). ●— 2nd year results; × — 3rd year results.

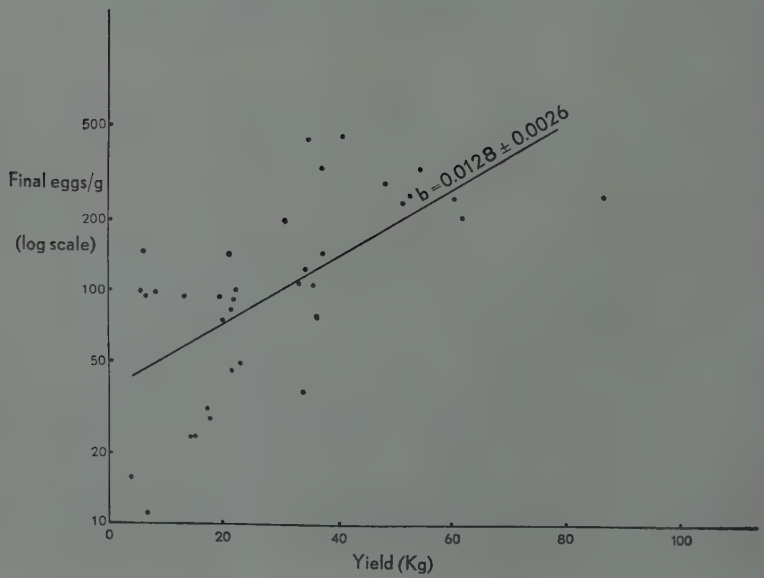

Fig. 4. The regression of log final egg population on yield (10 kg ≡ 2200 kg per hectare).

according to the frequency with which potatoes were grown. In the final year the average of the treatment I plots was 14.5 kg (1631 kg per hectare), and that of treatment II plots was 41 kg (4515 kg per hectare). In the second year the average of treatment III plots was 113.5 kg (12544 kg per hectare) compared with 78.5 kg (8780 kg per hectare) for treatment I plots.

Effect of ground keepers

An attempt was made in July to count volunteer potatoes in all non-potato plots but it proved impossible to get reliable counts because of weed growth, or cultivations. The highest number counted was 26 per 21 sq. m in a winter wheat plot, but even with this count the eelworm population dropped considerably.

DISCUSSION

The regression line calculated from untransformed data (second year results) shows that, over the range of initial populations studied, an increase of 50 eggs/g lowered the plot sample yield by 10 kg (about 2500 kg per hectare). If the point at which potato growing becomes unprofitable is taken as 12500 kg per hectare (5 tons per acre) the corresponding initial egg population is 27 and is equivalent to 31 "viable cysts" or 12 "full cysts" per 100 g of air dried soil. A "viable cyst" is defined as one containing one or more eggs and a "full cyst" as containing more than 50 eggs. In field population counts it is usually considered that cysts with less than 50 eggs can be ignored as they do not contribute much to the total egg population. This implies that egg counts are more nearly proportional to full cysts than to viable cysts.

Johnson & Thompson (1945) gave a figure for the "economic zero" of 50 viable cysts/100 g. This is rather higher than the figure of 31 "viable cysts" given here for a yield of 12 500 kg per hectare. However the yields on this field were probably low for reasons other than eelworm.

I thank the Statistics Department of Rothamsted Experimental Station who were consulted about the design of this experiment, J. R. Keyworth and J. C. Matthews who helped with the field work, and L. J. Coppock and W. E. Dant who helped with the eelworm counts and preparation of the figures.

ZUSAMMENFASSUNG

Ein Fruchtfolgeversuch auf mit Kartoffelnematoden (Heterodera rostochiensis *Woll.) verseuchtem Boden*

Ein Fruchtfolgeversuch wurde 1951 bis 1953 auf einem lehmigen Torfboden durchgeführt. Er bestand aus drei Blocks folgender 4 Versuchsreihen: 1. Kartoffeln jedes Jahr, 2. Kartoffeln in ungeraden Jahren, 3. Kartoffeln in 1952 und 4. Keine Kartoffeln. Die Veränderungen in den Zysten und Eipopulationen sind in Abb. 1 und 2 dargestellt. Veränderungen in der Eipopulation zeigen, dass bei geringer Anfangsverseuchung die Population gewöhnlich ansteigt und bei hoher Anfangsverseuchung gewöhnlich abfällt.

Die Regression der Ernte auf den log. der Anfangs-Eipopulation ist aus Abb. 3 zu entnehmen.

Für die Ergebnisse des zweiten Jahres betrug der Korrelationskoefficient (r) -0,739 (gesichert bei P = 0,01). Die Ergebnisse zeigen, dass eine Vermehrung von 50 Eier/g den Ertrag um etwa 2500 kg je ha erniedrigt und dass ein Ertrag von 12500 kg je ha (als unterste Grenze angenommen, bei der ein Kartoffelanbau unrentabel wird) einer Anfangs-Eipopulation von 27 Eier/g Boden entspricht.

Die Regression des log der endgültigen Eipopulation auf den Ertrag geht aus Abb. 4 hervor (Korrelationskoeffizient 0,649, gesichert bei P = 0,001).

Die Kartoffelerträge fielen schnell nach der Häufigkeit, mit der Kartoffeln angebaut werden, aber sie waren wahrscheinlich nicht wegen des Älchenbefalls niedrig.

REFERENCES

Chitwood, B. G. & Feldmesser, J. (1948). Golden nematode population studies. *Proc. helm. Soc. Wash.* **15**: 43-55.

Fenwick, D. W. & Reid, E. (1953). Population studies on the potato root eelworm, *Heterodera rostochiensis* Woll. *J. Helm.* **27**: 119-128.

Goffart, H. (1952). Ansteigen und Abklingen der Nematodenverseuchung und ihre Bewertung in Rübenanbau. *Zuckerrübenbau* **14**: 315.

Goodey, J. B. (1957). *Laboratory methods for work with plant and soil nematodes*. Tech. Bull No. 2 (H.M.S.O.) London 47 pp.

Hague, N. G. & Hesling, J. J. (1958). Population studies on cyst-forming nematodes of the genus *Heterodera*. *Proc. Linn. Soc.* **169**: 86-92.

Johnson, L. R. & Thompson, H. W. (1945). Potato root eelworm in Yorkshire. *Agriculture* **52**: 266-270.

Mai, W. F. (1954). Changes in the viable golden nematode population of the soil while growing potatoes every year. *Phytopathology* **44**: 497.

Oostenbrink, M. (1950). Het aardappelaaltje (*Heterodera rostochiensis* Woll.). Een gevaarlijke parasiet voor de eenzijdige aardappelcultuur. *Versl. Med. Plziekt. Dienst Wageningen* **115**.

Schmidt, J. (1954). Zur Populationsdynamik des Kartoffelnematoden. *Wiss. Z. Univ. Rostock; Math. Naturw. Reihe* **4**: 187-190.

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THE RESPONSE OF POTATO ROOT EELWORM, *HETERODERA ROSTOCHIENSIS* WOLLENWEBER AND BEET EELWORM, *H. SCHACHTII* SCHMIDT TO ROOT DIFFUSATES OF SOME GRASSES, CEREALS AND OF *TAGETES MINUTA*

BY

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Three experiments failed to produce evidence that root diffusates from cereals and grasses stimulate eggs of *Heterodera rostochiensis* to hatch *in vitro*. Diffusate from *Tagetes minuta*, tested in one experiment, neither stimulated nor inhibited hatch. Diffusate from cereals, grasses and *Tagetes minuta* differed very little in effect from soil water. Diffusate from rye and perennial rye grass did not stimulate cysts of *H. schachtii*. The experiment emphasized the need to test diffusates over a range of dilutions.

Triffitt (1932, 1934) studied the influence of root diffusates from ten commonly grown grasses and cereals on the emergence of larvae from cysts of the potato root eelworm, *Heterodera rostochiensis* Woll., then thought to be a strain of *H. schachtii* Schmidt. Her experiments suggested that root diffusates of *Poa trivialis* L., *P. pratensis* L., *Lolium perenne* L. and *Dactylis glomerata* L. stimulated the larvae to hatch *in vitro*, although to a lesser extent than potato root diffusate. The *Poa spp.* hatched from one-third to almost five-sixths as many larvae as potato root diffusate. From these results and from those of some pot and field experiments, Triffitt concluded that the meadow grasses might be used as 'enemy' plants against potato root eelworm, because the grasses are not hosts of this nematode. Schmidt (1930) also concluded from hatching tests that root diffusates of cereals stimulated emergence of beet eelworm and suggested that further work might show their possible usefulness as 'enemy' plants, and this term was applied to maize and rye by both Nebel (1926) and Molz (1932).

In contrast, Oostenbrink (1950), using *H. rostochiensis*, showed that the hatching activity of root diffusate from thirty different plants was the same as tap water and produced only one tenth of the number of larvae hatched by potato-root diffusate. Similar results were obtained by Stelter (1958).

The conflicting evidence was therefore checked in three experiments. Altogether, the diffusates of twelve grasses and five cereals were compared with potato root diffusate and soil leachings. In addition, the effect of rye and rye grass diffusates was tested on cysts of *H. schachtii*, and that of *Tagetes minuta* on cysts of *H. rostochiensis*.

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METHODS

Cysts of *H. rostochiensis* for the first and second experiment were extracted in bulk from infested land and, for the third, cysts of *H. rostochiensis* and *H. schachtii* were raised in pot-cultures in the previous year.

Diffusates for the first and second experiments were obtained by sowing 18 cm diam. pots with cereal and grass seeds, three pots to each species. Diffusate was collected at intervals of about 7 days and the yield from each pot on each occasion was stored in separate bottles at 2° C, so that the hatch in diffusate from individual pots could be traced. In the first experiment, 200 ml of diffusate was collected from each pot at each leaching. In the second, pots were first moistened and then 200 ml of tap water added, the leachate collected and poured back through the pot four times. In both experiments soil leachings were produced in the same way as the cereal and grass diffusates but potato root diffusate was drawn from a separate stock.

Diffusate for the third experiment was obtained from ten 15 cm diam. pots. Pots were leached after the plants had been growing for 17-20 days by adding just sufficient tap water to produce 15 ml leachate per pot. Potato root diffusate and soil leachate were produced in the same way. The diffusates of each species were bulked, filtered and stored at 5° C.

The techniques used in hatching tests followed closely those of Fenwick & Widdowson (1958), as described by Goodey (1957). In the first and second experiment four replicate batches of 100 cysts were used to test the diffusate from each pot. In the third experiment the diffusates and soil leachate were tested at full strength and diluted 1/4, 1/16, 1/64 in water. Four replicate batches of 100 cysts were used at each dilution. In all three experiments diffusates were changed at weekly intervals and numbers of hatched larvae were estimated from counts of 1 ml aliquots drawn from suspensions made up to 10 ml in the first and second experiments and 25 ml in the third.

Potting soil for the first and second experiments was drawn from an infested field. In the second experiment, cysts were extracted after the cereals and grasses had been grown and cyst contents were estimated on batches of 100 cysts by the hypochlorite technique (Fenwick 1942). These were compared with cyst contents of unplanted potting soil used to produce soil leachate. In the third experiment sterilised potting compost was used.

RESULTS

The results of the first experiment (Table I) contained some unsatisfactory features. In some instances there were gross differences between diffusates collected from pots sown with the same cereal or grass. Thus for some species the mean for the three pots is inflated by these figures and gives the impression that some grasses and cereals cause a small but appreciable hatch compared with soil leachings or potato root diffusate. However, if the anomalous results are excluded and the means calculated only from the lower hatches, which predominate, these means

are very little different from those of soil water and do not approach that of potato root diffusate. These adjusted means are shown in brackets in Table I. These discrepancies, traceable back to individual pots, may possibly have been caused by the growth of small seedlings of *Solanum nigrum* L. hidden by the grass and cereals. In the second and third experiments great care was taken to ensure that all cysts were treated similarly before the test, that glass was meticulously clean and that pots were free from weeds.

TABLE I

Emergence of larvae from cysts of Heterodera rostochiensis. *Cumulative hatches per 100 cysts after 28 days in diffusate at 25° C. Means of twelve replicates.*

	Pots leached after plant growth for: —		
	17 days	20 days	28 days
Grasses			
Alopecurus pratensis	23	58	35
Arrhenatherum elatius	64	66	105
Dactylis glomerata	721(59)	213(37)	322(37)
Festuca pratensis	590(116)	495(142)	786(273)
Festuca rubra	100	66	96
Lolium multiflorum	867(152)	381(60)	594(15)
Lolium perenne	95	56	41
Phleum pratense	180	104	110
Poa pratensis	51	57	51
Poa trivialis	211(44)	454(47)	306(88)
Mean	*335(89)*	*195(70)*	*245(85)*
Cereals			
Maize *Zea mays*	1710(443)	419(159)	208(120)
Oat *Avena sativa*	301	249	268
Rye *Secale cereale*	68	29	139
Wheat *Triticum sativum*	1019(303)	362(85)	173(35)
Mean	*774(279)*	*265(130)*	*197(140)*
Potato			
Undiluted diffusate	*3843*	*2263*	*2795*
Control			
Soil leachings	*163*	*59*	*60*

In the second experiment (Table II), the diffusates from cereals and grasses after 14-15 days growth gave an emergence from cysts of the same order as that from soil water and about one-eightieth of that from diluted potato root diffusate. The results were similar after 21-22 days growth, but now cereals and grasses gave a hatch about one-tenth of that given by potato root diffusate and about two and a half times that of soil water. As these tests were not done in a series of dilutions, the possible inhibitory action of soil water could not be taken into

account. It is clear, however, that diluted potato root diffusate was an effective hatching agent but the diffusates of grasses and cereals were not.

TABLE II

Emergence of larvae from cysts of Heterodera rostochiensis

	Pots leached after plants growth for: —		
	14-15 days *(Cumulative hatch 15 days)	21-22 days *(Cumulative hatch 20 days)	Mean larval content of cysts from the pots
Grasses			
Alopecurus pratensis	55	369	99
Arrhenatherum elatius	101	457	105
Dactylis glomerata	80	452	105
Festuca elatior	127	304	110
Festuca pratensis	87	482	95
Festuca rubra	66	407	99
Lolium multiflorum	79	364	106
Lolium perenne	93	374	97
Phleum pratense	75	647	81
Poa pratensis	136	276	96
Poa trivialis	135	430	108
Mean	*94*	*415*	*100*
Cereals			
Oat *Avena sativa*	110	302	95
Barley *Hordeum sativum*	224	295	97
Rye *Secale cereale*	115	294	75
Wheat *Triticum sativum*	147	385	81
Maize *Zea mays*	53	454	95
Mean	*130*	*346*	*89*
Potato			
Diffusate diluted seven times	*9064*	*4198*	
Control			
Soil leachings	*120*	*149*	*93*

* Cumulative hatch from 100 cysts, means of twelve replicates.

The results from the third experiment (Table III) confirm those of the first and second. In this, a series of dilutions was used for all treatments. The inhibitory action of soil water can be clearly seen and there is also evidence of inhibition by the undiluted potato and beet root diffusates (see also Winslow, 1954, and Hague, 1958). The effects of diffusate from cereals, grasses and *Tagetes minuta* are closely comparable with each other and with soil water. The root diffusates of these plants, therefore, appear to have neither inhibitory nor stimulatory action.

The larval content of cysts extracted from the pots of infested soil used in the production of grass and cereal diffusates was estimated in the second experiment. The third column of Table II shows that the numbers of eggs per cyst did

not differ significantly from the controls (soil without plants), confirming the results of the hatching tests.

TABLE III

Emergence of larvae from cysts of Heterodera. *Mean cumulative hatches from four batches of 100 cysts after 21 days at 25° C.*

	Dilutions			
	Undiluted	1 : 4	1 : 16	1 : 64
H. ROSTOCHIENSIS				
Grasses				
Alopecurus pratensis	200	256	519	1319
Arrhenatherum elatius	63	138	594	2419
Cynosurus cristatus	119	238	1013	2019
Dactylis glomerata	106	131	400	1519
Festuca elatior	100	194	1131	2363
Festuca pratensis	119	156	419	906
Lolium multiflorum	200	206	594	850
Lolium perenne	175	188	1150	1975
Phleum pratense	94	250	350	631
Poa pratensis	56	394	806	1419
Poa trivialis	81	163	231	363
Mean	*119*	*189*	*655*	*1435*
	(4)	(7)	(24)	(52)
Cereals				
Oat *Avena sativa*	156	217	481	806
Barley *Hordeum sativum*	75	156	394	—
Rye *Secale cereale*	150	181	831	1144
Wheat *Triticum vulgare*	131	181	263	750
Mean	*128*	*184*	*492*	—
	(5)	(7)	(18)	—
Tagetes minuta	*87*	*225*	*988*	*2269*
	(3)	(8)	(22)	(83)
Soil leachings	*156*	*313*	*1581*	*2219*
	(6)	(11)	(36)	(81)
Potato root diffusate	*5169*	*10419*	*10600*	*6894*
	(189)	(380)	(387)	(252)
Distilled water		*2738*		
		(100)		
H. SCHACHTII				
Rye grass *Lolium perenne*	2744	2244	2087	3556
Rye *Secale cereale*	1363	1787	3281	2619
Mean	*2054*	*2016*	*2684*	*3088*
	(85)	(84)	(111)	(128)
Sugar beet *Beta vulgaris*	*17419*	*15725*	*10012*	*7769*
	(720)	(650)	(414)	(321)
Distilled water		*2419*		
		(100)		

Note. The figures in brackets are percentages using distilled water as the standard (100%).

DISCUSSION

The lack of agreement between Oostenbrink's, Stelter's, and our results and those of Triffitt is difficult to explain. Techniques have improved since her tests but she was well aware of the difficulties of working with cysts of *Heterodera*. To reduce error she used batches of 300 cysts in hatching experiments and, although treatments were not replicated in experiments, each experiment was repeated several times with similar results.

In field experiments, Triffitt found that the grasses decreased cyst contents by 23% compared with 2% in the fallow controls. Franklin (1937) showed that maize grown on land infested with potato root eelworm decreased the population by 35% and lupins by 16%. None of these decreases is greater than the 40-50% which might be expected under fallow. Mai & Lownsbery (1952) also claimed that potato root eelworm is affected more by some grasses than by other non-host crops, but they estimated population changes by counting white cysts on the roots of subsequent potato crops, a method unlikely to estimate the population level reliably. The effect of grasses in the field needs re-examining, using the improved techniques of population estimation now available, before conclusions can be drawn. At present there is no reliable evidence that grasses or cereals bring the population of potato root eelworm below the level obtained by fallowing.

The response of *H. rostochiensis* to the root diffusate of *Tagetes minuta* was similar to that in cereal and grass diffusates and in soil leachings. There was no evidence that the diffusate contained enough of the nematicidal principles in *Tagetes* roots (Uhlenbroek & Bijloo, 1958, 1959) to affect the hatch of *H. rostochiensis*.

The difficulties experienced in the first experiment show the need for exceptional care in hatching tests and the third experiment shows the desirability of working with a suitable range of dilutions.

We thank Elizabeth Widdowson and Ann Seabrook for assistance with the experiments and F. G. W. Jones for help with the script and tables.

ZUSAMMENFASSUNG

Die Reaktion des Kartoffelälchens, Heterodera rostochiensis *Wollenweber und des Rübenälchens,* Heterodera schachtii *Schmidt, auf Wurzeldiffusate von Gräsern, Getreide und* Tagetes

Die Schlüpfwirkung der Wurzeldiffusate von Getreide und Gräsern auf Zysten von *Heterodera rostochiensis* wurde *in vitro* in drei Versuchen geprüft. Diffusate wurden durch Auslaugen von mit geeigneten Pflanzen besäten Töpfen nach 7 Tagen erhalten. Beim zweiten und dritten Versuch kam die übliche Technik der Gewinnung von Diffusaten zur Anwendung. In zweiten Versuch wurde Boden, der mit *H. rostochiensis* infiziert war, benutzt und der Zysteninhalt am Ende des Versuchs geschätzt. Das Standard-Schlüpfverfahren nach Fenwick & Widdowson (1958) wurde in mehreren Schalen mit 100 Zysten verwendet. Im zweiten Versuch wurde noch grössere Sorgfalt auf Vorbehandlung der Zysten, Reinigung der Gläser und Entfernung von Unkräutern aus den Töpfen gelegt. Im dritten Versuch kamen Wurzeldiffusate und Bodenauswaschungen in einer Reihe von Lösungen gegenüber den Zysten zur Anwendung. Die Ergebnisse zeigen, dass Diffusate von Getreide, Gräsern und *Tagetes minuta* keinen Einfluss auf *H. rostochiensis in vitro* haben. Die Schlüpfzahlen waren mit denen vergleichbar, die durch Bodenauswaschungen erzielt wurden und lagen unter denen von Kartoffelwurzeldiffusat. In ähnlicher Weise hatten Roggen- und Gras-

diffusate auf *H. schachtii* keinen Einfluss. Das Wachstum von Gräsern in Töpfen mit verseuchtem Boden reduzierte im Vergleich zur Brache nicht die Zahl der Larven in den Zysten von *H. rostochiensis*. Die Ergebnisse von Triffitt (1932; 1934), bei denen sich eine Schlüpfwirkung auf Gräserdiffusate ergab, sind schwer zu erklären. Auf Sorgfalt und Verwendung geeigneter Lösungen ist Wert zu legen.

REFERENCES

Fenwick, D. W. (1942). The degree of *Heterodera* infectivity of soil and its determination. *J. Helm.* **20**, 50-66.

Fenwick, D. W. & Widdowson, E. (1958). The conduct of hatching tests on cysts of the potato-root eelworm, *Heterodera rostochiensis*. *J. Helm.* **32**, 125-134.

Franklin, M. T. (1937). The effect on the cyst contents of *Heterodera schachtii* of the cultivation of maize on potato sick land. *J. Helm.* **15**, 61-68.

Goodey, J. B. (1957). Laboratory methods for work with plant and soil nematodes. *Tech. Bull. Minist. Agric., Lond.* 2, 3rd ed., (H.M.S.O.), 47 pp.

Hague, N. G. (1958). The concentration of potato-root diffusate under reduced pressure. *Nematologica* **3**, 149-153.

Mai, W. F. & Lownsbery, B. F. (1952). Crop rotation in relation to the golden nematode population of the soil. *Phytopathology* **42**, 345-347.

Molz, E. (1932). Die Bekämpfung des Rübennematoden mittels des Chlorkalk-Aktivierungsverfahrens. *Dtsch. Landw. Pr.* **59**, 371-372.

Nebel, B. (1926). Zur Frage der Nematodenbekämpfung. *Zuckerrübenbau* **8**, 93-101.

Oostenbrink, M. (1950). Het aardappelaaltje (*Heterodera rostochiensis* Wollenweber) een gevaarlijke parasiet voor de eenzijdige aardappelcultuur. *Versl. PlZiekt. Dienst Wageningen* **115**: 230 pp. (144-150).

Schmidt, O. (1930). Sind Rüben- und Hafer-Nematoden identisch? *Pflanzenbau* **3**, 420-464.

Stelter, H. (1958). Untersuchungen über den Kartoffelnematoden *Heterodera rostochiensis* Wollenweber, IV. Der Einfluss von Mais (*Zea mays*) auf den Kartoffelnematoden. *NachrBl. Deutsch. PflSchDienst Berlin* **12**: 133-135.

Triffitt, M. J. (1932). Preliminary note on the use of root excretions as a method of controlling the nematode *Heterodera schachtii*. *J. Helm.* **10**, 181-182.

—— (1934). Experiments with root excretions of grasses as a possible means of eliminating *Heterodera schachtii* from infected soil. *J. Helm.* **12**, 1-12.

Uhlenbroek, J. H. & Bijloo, J. D. (1958). Investigations on nematicides. I. Isolation and structure of a nematicidal principle occurring in *Tagetes* roots. *Rec. Trav. chim. Pays-Bas* **77**, 1004-1009.

—— & —— (1959). Investigations on nematicides. II. Structure of a second nematicidal principle isolated from *Tagetes* roots. *Rec. Trav. chim. Pays-Bas* **78**, 382-390.

Winslow, R. D. (1954). The hatching responses of some root eelworms of the genus *Heterodera*. *Ann. appl. Biol.* **43**, 19-36.

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THE EFFECTS OF RED BEET AND OF *HESPERIS MATRONALIS* L. ON A POPULATION OF *HETERODERA SCHACHTII* SCHM.

BY

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The effect on a population of beet eelworm of growing *Hesperis matronalis* was tested in microplots in 1956. For comparison, two other treatments were used: fallow, and red beet. Linear regressions of final on initial eelworm densities were made, with modification of the usual analysis to allow for the errors of measurement in the initial population. The rate of decay for eggs with *H. matronalis* increased with higher initial egg density, but was never sufficiently greater than the rate with fallow to be worthwhile for controlling infestations of beet eelworm. Red beet acted as a trap-crop in this year; few if any new cysts were formed, and the rate of decay was similar to that of *H. matronalis*.

Heterodera schachtii Schm. (beet eelworm) does not form cysts on the roots of the Crucifer *Hesperis matronalis* L. (Winslow, 1954), although the roots of this plant secrete a substance which stimulates the hatch of the eelworm. (Den Ouden, 1954). Thus if *Hesperis matronalis* could be grown as a crop in the field, it might control beet eelworm. Den Ouden (1956), who considered that it was a suitable catch crop, found in both pot and field-plot experiments that the eelworm population decreased by about two-thirds in one season under *H. matronalis,* compared with about one-third under fallow or with a non-host.

The following microplot experiment was made to compare the effect of *H. matronalis,* red beet and fallow on the density of beet eelworm in the soil.

PROCEDURE

A block of 56 microplots (Jones, 1956) was used, filled with a black fen soil from Oxlode, Isle of Ely. In 1955, after cropping with three host plants at various population densities (Jones, 1957) [1]), one soil sample for the estimation of eelworm density was taken from each microplot, and examined at the Nematology Department, Rothamsted. The microplots were left fallow over the winter. On the 2nd May, 1956, 23 microplots were sown with red beet (Detroit Globe) to give a final density of 20 plants per microplot (equivalent to 120,000 plants/acre), and 23 were sown with *H. matronalis* to give a density of 6 plants per microplot (equivalent to 36,000 plants/acre). The other ten microplots were left fallow.

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[1]) Jones (1957) describes experiments made with these microplots in 1955. On p. 261 the year is incorrectly stated as 1956.

For the allocation of treatments to microplots, the microplots were arranged in order of initial eelworm egg density, and treatments were allotted at random within ten groups of microplots with similar egg densities. This ensured similar ranges of initial eelworm egg density for all three treatments. But it also meant that treatments were not distributed randomly over the block of 56 microplots. This is a disadvantage of the microplot technique. However, there was no apparent bias in the distribution of treatments between microplots.

The crops were lifted on September 18, 1956, and soil samples taken on November 3, 1956. For both these samples and those taken in 1955, each sample consisted of twenty uniformly-spaced borings taken to a depth of 20 cm with a 1.8 cm diameter semi-cylindrical sampler. Two 100 g sub-samples were taken

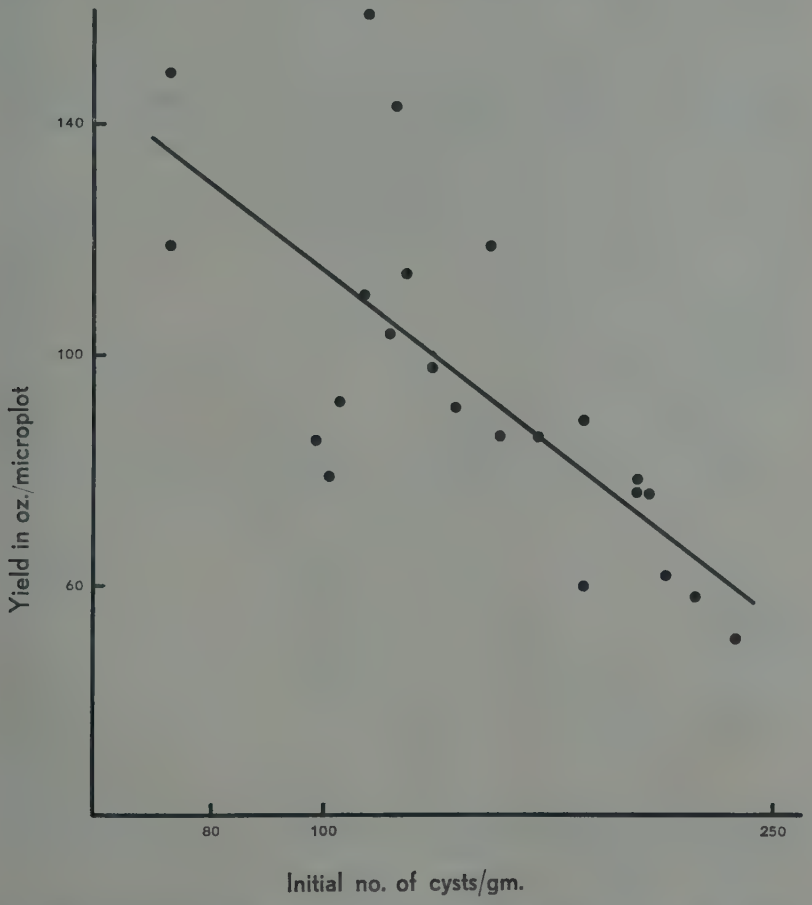

Fig. 1. The regression of yield of roots of red beet on the logarithm of the initial number of eggs/gm. soil.

from each sample. Cysts were collected by flotation, and egg counts made from suspensions of the squashed cysts (Goodey, 1957).

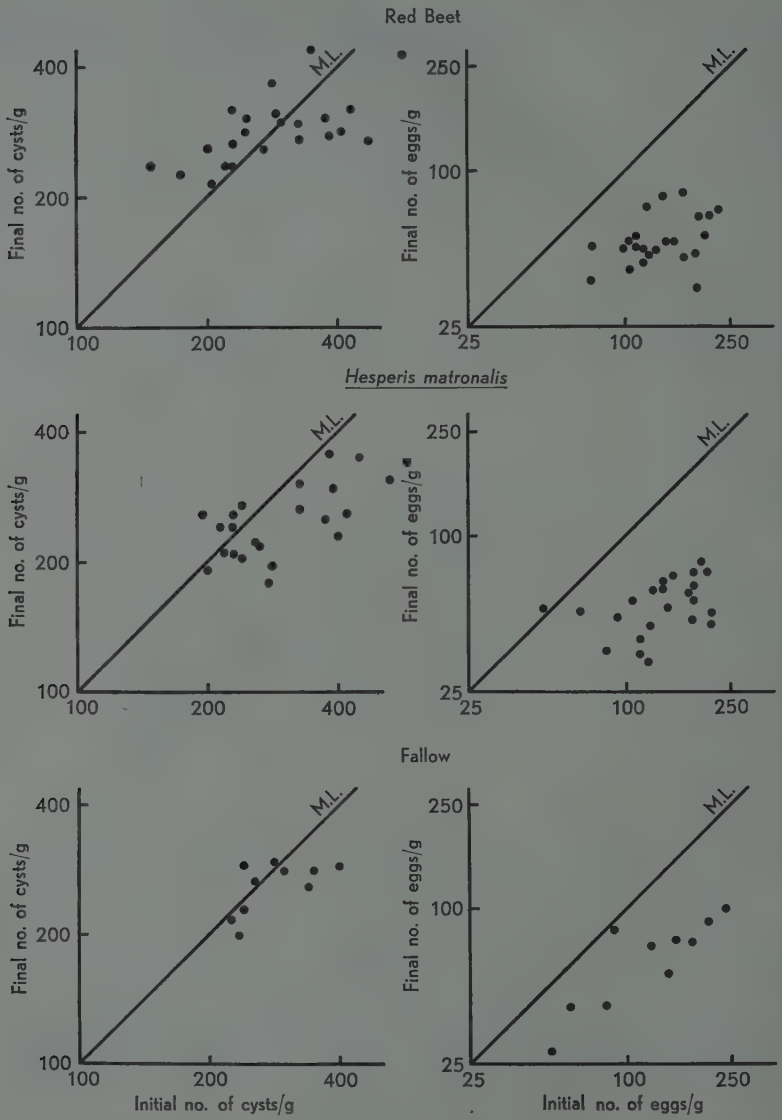

Fig. 2. Graphs of the initial and final cyst and egg densities with fallow, red beet and *Hesperis matronalis*, showing the maintenance levels (M.L.).

RESULTS

Plant Growth

At first the *H. matronalis* grew slowly, but by the time that the crops were lifted the plants were well established, with most of the ground covered by the leaves.

The red beet grew quite well considering the late date of sowing. A linear regression of yield on the logarithm of the initial eelworm population was obtained for both roots and tops. That for roots is shown in Fig. 1. The slope was significantly different from zero at the 0.001 probability level ($b = -151.8 \pm 31.2$). This value for the regression coefficient meant that for a two-fold increase of initial eelworm density, the yield of roots decreased by 1295.5 g (45.7 oz.).

Eelworm density

The logarithmic transformation of egg and cyst densities was used for all calculations (Moriarty, 1960). The values obtained for initial and final densities are shown in Figure 2. Linear regressions were most suitable: deviations from linearity were tested with the data for cyst counts with red beet by fitting a quadratic regression. The quadratic component was highly insignificant. The three regression lines for final cyst density on initial cyst density are shown in Fig. 3.

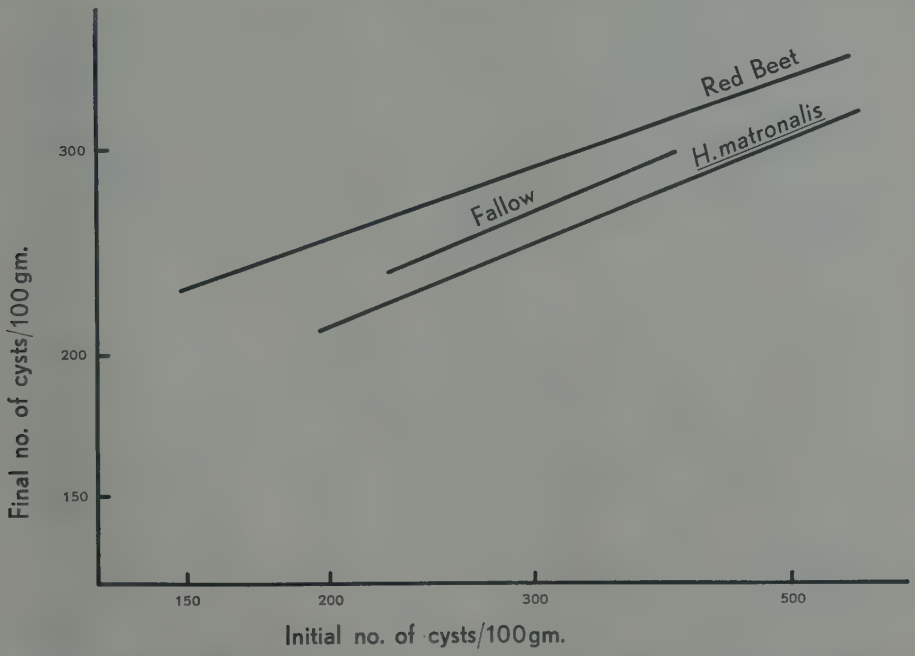

Fig. 3. The regressions of cyst densities after fallow, red beet and *Hesperis matronalis* on the initial cyst densities.

There is no significant difference between them in either the slope or origin. The estimated value of the slope for all three treatments combined is 0.36 ± 0.07.

The three regression lines for final egg density on initial egg density are shown in Fig. 4. The regressions for red beet and *H. matronalis* are almost identical. The estimated values of the regression coefficients and their standard errors for fallow, and for red beet and *H. matronalis* combined, are shown in Table I. They are significantly different at the 0.05 probability level ($t = 2.405$).

DISCUSSION

With the standard methods of calculating regression coefficients one assumes that the values of the independent variable are known exactly (Fisher, 1958). In this experiment the estimates of the initial egg and cyst densities have an appreciable error (Moriarty, 1960), which affects the estimated value of the regression coefficient. To obtain a better estimate, information is needed about some or all of the following quantities: the correlation between the errors of measurement in the two variables, and the variance of the errors of measurement for both variables. Acton's (1959) method was used to recalculate the regression coefficient, assuming the correlation between errors to be zero, and the standard deviations in errors of measurement of initial and final densities to be identical.

The regression coefficient for fallow is just significantly different from 1 at the 0.05 probability level, and after the correction for errors in the initial density is not so. For the effect of red beet and *H. matronalis* on egg density however, the regression coefficient is significantly less than 1 (Table I). The regression coefficient for final cyst density on initial cyst density is also significantly less than 1, and there is no significant difference between treatments. If the rate of decay was independent of the initial eelworm density, then with the logarithmic transformation the regression coefficient would not be significantly different from 1.

TABLE I

Values for the linear regression of final egg density on initial egg density. All values are based on the logarithmic transformation.

Treatment.	Range of initial egg densities (1955)	Range of final egg densities (1956)	Mean initial egg density	Mean final egg density	Regression coefficient (b) ± standard error	Value of b allowing for errors in initial density
Fallow	1.71-2.37	1.45-2.00	2.07	1.79	0.66 ± 0.15	0.75
Red beet and *Hesperis matronalis*	1.69-2.36	1.51-1.91	2.13	1.73	0.26 ± 0.09	0.39

Jones (1956) found, in 2-acre field plots, that the rate of decay for populations of beet eelworm in the absence of host crops is independent of the population level; Den Ouden (1956) also found no indication that the rate of decay of beet

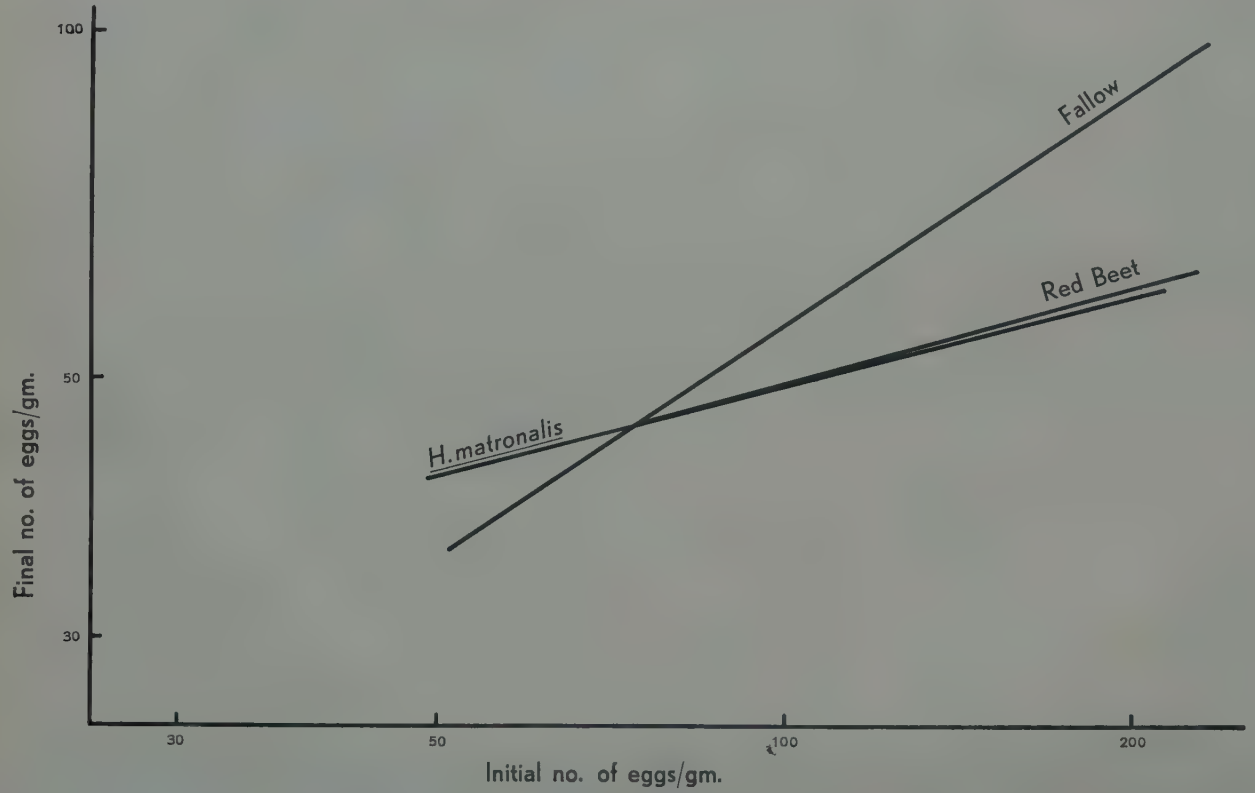

Fig. 4. The regressions of egg densities after fallow, red beet and *Hesperis matronalis* on the initial egg densities.

eelworm in the presence of *H. matronalis* varied with the population level. In my experiment however, within the tested range of eelworm density, the rate of decay increased as the initial eelworm density increased. This variation of decay rate with initial egg density was probably caused by varying responses to diffusate, because there was little evidence of it under fallow: the denser populations of eggs had a higher proportion that was stimulated to hatch by diffusate. It is possible that the larger initial populations contained a higher proportion of older eggs.

The result obtained with red beet was surprising, as it has been shown to be a good host plant (Jones, 1956). The regression of yield on initial eelworm density (Fig. 1) is similar to those obtained by Jones (1956), and shows that the larvae attacked the roots. The cyst counts (Fig. 3) show that few if any eelworms completed their life cycle. Jones (1950) found that in field conditions beet eelworm takes from one to two months to complete its life cycle. The weather was abnormally dry until June, and later in the year the temperatures were very low. This combination of factors probably resulted in a slow initial invasion, and slow rate of development. In fact the red beet acted as a trap crop.

The decay rate of beet eelworm under fallow was 48 %, which agrees with other estimates of the decay rate of eggs (Hijner, 1956; Jones, 1956), although Den Ouden (1956) obtained a decay rate of only 38 % under wheat and barley. With red beet and *H. matronalis,* using the corrected regression coefficient of 0.39, the decay rate at an initial density of 229 eggs/g was 67 %. This is a fairly severe infestation, and at lower densities the decay rate was also lower: the decay rate at an initial density of 90 eggs/g was 37 %. Den Ouden (1956) also obtained a decay rate with *H. matronalis* of 66 %. The evidence suggests that populations of beet eelworm are decreased slightly more by *H. matronalis* than by fallow or non-host crops, but this alone is insufficient to render it a useful agricultural crop. Winslow (1955) reached a similar conclusion for the use of *Solanum nigrum* as a trap crop for *Heterodera rostochiensis.*

I am indebted to Mr. F. G. W. Jones for the figures from the 1955 soil samples, and to Dr. R. C. Campbell for advice on the statistical treatment of the data. I am grateful to Mr. P. Halfpenny for assistance with this work. The work was financed by the Sugar Beet Research and Education Committee.

ZUSAMMENFASSUNG

Der Einfluss von Roter Rübe und Hesperis matronalis *L. auf eine Population von* Heterodera schachtii *Schm.*

Der Einfluss von *Hesperis matronalis*-Pflanzen auf eine Population von *Heterodera schachtii* wurde 1956 in Kleinparzellenversuchen im Vergleich zu Brache und Roter Rübe geprüft. Lineare Regressionen der letzten Älchendichte zur Anfangsverseuchung wurden mit einer Veränderung der gewöhnlichen Analyse vorgenommen, um Messfehler bei der Anfangspopulation auszugleichen. Die Abnahme an Eiern nahm bei *Hesperis matronalis* mit höherer Anfangseimenge zu, war aber nie grösser als die Abnahme nach Brache und daher für die Bekämpfung des Rübennematoden unbedeutend. Rote Rübe wirkte als Fangpflanze; einige neue Zysten wurden gebildet. Der Rückgang in der Verseuchung war ähnlich wie bei *Hesperis matronalis.*

REFERENCES

ACTON, F. S. (1959). *Analysis of straight-line data.* New York, Wiley, 267 pp.

FISHER, R. A. (1958). *Statistical methods for research workers.* Edinburgh, Oliver and Boyd, 13th edn., 356 pp.

GOODEY, J. B. (1957). *Laboratory methods for work with plant and soil nematodes.* Tech. Bull. 2, Minist. Agric., London, H.M.S.O. 3rd edn., 47 pp.

HIJNER, J. A. (1956). Analysis of soil samples for beet eelworm cysts. *Rapp. Inst. Int. Rech. Betteravières, XVIe Assembl.,* Bruxelles, 129-139.

JONES, F. G. W. (1950). Observations on the beet eelworm and other cyst-forming species of *Heterodera. Ann. appl. Biol.* **37**, 407-440.

—— (1956). Soil populations of beet eelworm *(Heterodera schachtii* Schm.) in relation to cropping. II. Microplot and field plot results. *Ann. appl. Biol.* **44**, 25-56.

—— (1957). Soil populations of beet eelworm *(Heterodera schachtii* Schm.) in relation to cropping. III. Further experiments with microplots and with pots. *Nematologica,* **2**, 257-272.

MORIARTY, F. (1960). Laboratory errors associated with the estimation of the population density of *Heterodera* species in the soil. *Ann. appl. Biol.* **48**, 665-680.

OUDEN, H. DEN (1954). Het bietencystenaaltje en zijn bestrijding. III. Lokplanten-onderzoek. *Med. Inst. Rat. Suikerprod.* **24**, 141-155.

—— (1956). The influence of hosts and non-susceptible hatching plants on populations of *Heterodera schachtii. Nematologica,* **1**, 138-144.

WINSLOW, R. D. (1954). Provisional lists of host plants of some root eelworms (*Heterodera* spp.). *Ann. appl. Biol.* **41**, 591-605.

—— (1955). Experiments on the control of the potato root eelworm by trap cropping with black nightshade. *Plant Path.* **4**, 139-140.

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THE ORIENTATION OF *DITYLENCHUS DIPSACI* TO PHYSICAL STIMULI

BY

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In a temperature gradient of 2-30° C over 15 cm, *D. dipsaci* aggregates at about 10° C. In a moisture gradient the nematodes aggregate at the end with a high moisture content and, in a particle size gradient they collect in the fine particles at one end of the gradient. Light, gravity and water percolation do not affect the orientation of *D. dipsaci* although passive directional movement may occur with the last two stimuli.

Most nematodes live in water films in the soil for at least part of their life-cycle where they are subjected to several physical stimuli which might influence their behaviour and movements. Although work on the orientation of both phyto- and zooparasitic nematodes is sparse and the physical conditions of the experiments are often ill-defined, there are enough data to show fundamental similarities between the two groups. Discussion of orientation will, therefore, embrace the Nematoda as a whole. This investigation attempts to answer two questions: do physical factors have any influence on the orientation and aggregation of the stem eelworm, *Ditylenchus dipsaci* (Kühn), and if so, how is this accomplished? The effect of temperature, moisture, particle size, light, water percolation and gravity are considered. The influence of oxygen and carbon dioxide gradients have not been studied because it is considered that, as yet, there is no precise way of measuring their concentrations in soil water films. Fourth stage larvae of *D. dipsaci* (oat race) were recovered from dried oat material in a mistifier apparatus and, before each experiment, they were placed in a small drop of water to ensure that they were as active as possible. The infested oats were collected from a plot at Rothamsted in August, 1959, dried, and used the following year. Experiments other than those studying temperature were done at 18-20°C.

TEMPERATURE

Method

A temperature gradient was set up in a polythene tube, 15 cm long and 6 mm internal diameter, containing water-saturated sand of particle diameter 250-500 μ (Fig. 1). Six thermocouples were used to measure temperature along the tube which was placed in a boiling tube containing steel wool which, at one end, extended into a perforated tin containing a 5 watt bulb. The other end of the

boiling tube was inserted into a vacuum flask containing crushed ice. The exposed part of the boiling tube was surrounded by cotton wool contained in a cardboard tube. The electric bulb was connected to an intermittent switch which, after adjustment, enabled the temperature in the perforated tin to be kept constant. A constant temperature gradient from about 2 to 30° C could be maintained in the sand for 48 hours. Nematodes were introduced into the required spot through a hole bored in the tube; the hole was then sealed with plasticene. During the experiment, readings were taken to ensure that the temperature gradient was steady and, at the end of the experiment the polythene tube was removed, cut into 1 cm sections and the nematodes in each section counted. It was necessary to use saturated sand because at any other moisture content, moisture gradients would arise owing to temperature differences along the tube.

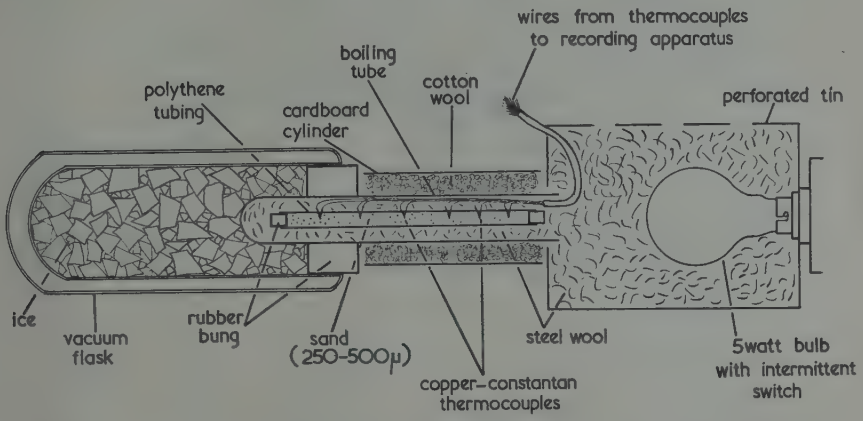

Fig. 1. Apparatus for maintaining a temperature gradient in a polythene tube (15 cm long) containing water saturated sand.

To measure mobility at different constant temperatures, polythene tubes 6 cm long and 5 mm internal diameter were filled with saturated sand and placed horizontally. Nematodes were introduced at one end and after 5 hours the percentage which had migrated more than 3 cm was assessed.

Results

About 200 *D. dipsaci* were introduced into the temperature gradient at the 20° C point, 5 cm from the hot end of the tube. After 5 hours there was an apparent aggregation at about 10° C (Fig. 2a). In a second experiment nematodes introduced at about 10° C were distributed randomly about this temperature after 5 hours (Fig. 2b). To test the possibility that 10° C is a temperature "preferendum" *D. dipsaci* were inoculated into the gradient at about 15° C and their distribution determined after 1, 5 and 20 hours in separate experiments. In all

three experiments there were two distribution peaks, the first where the nematodes were introduced into the sand and the second at about 10° C (Fig. 3). The results suggest that *D. dipsaci* has a temperature "preferendum" of 10° C to which it will move in a temperature gradient. Nematodes were introduced at 4° C in the

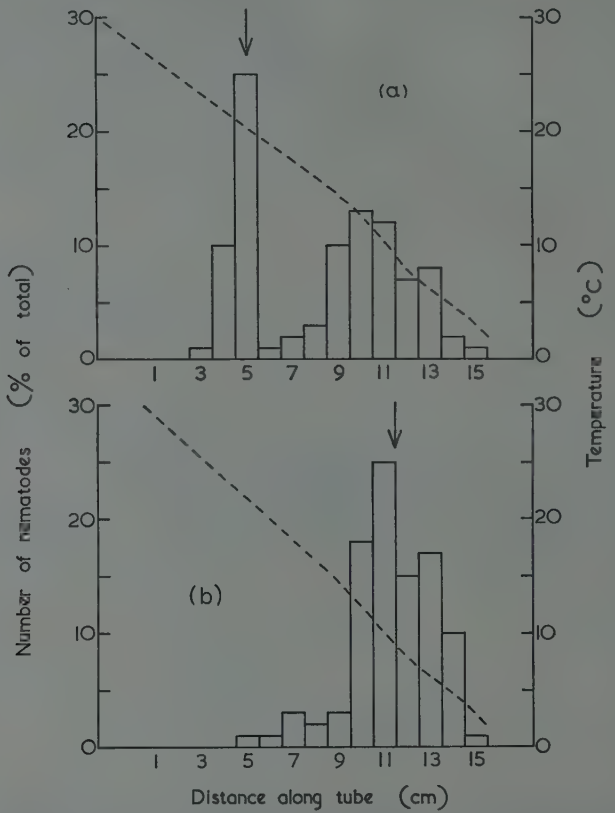

Fig. 2. Distribution of *D. dipsaci* in a 15 cm tube of saturated sand after 5 hours in a temperature gradient. The dotted line shows the temperature gradient and the arrow the point where the nematodes were inoculated into the sand at (a) 20° C and (b) 10° C.

gradient and their distribution was determined after 5, 20 and 48 hours in separate experiments (Fig. 4). There was a slight spread towards the hot end of the gradient but aggregation was apparent only in the 20 hour experiment where a peak occurred at 7-8° C. It is likely that the mobility of *D. dipsaci* at 4° C is so low that any directional movement would be very slow.

MOISTURE

Method

Moisture gradients were obtained by exerting a suction on saturated sand (250-500 μ diameter) contained in vertical polythene tubing 10 cm long and 5 mm

internal diameter (Wallace 1960). The relationship between suction and moisture content was obtained from the moisture characteristic (Wallace 1958a) and, by using different suctions, different moisture gradients were obtained. The eelworms were introduced into the sand through a hole in the tube. At the end of the

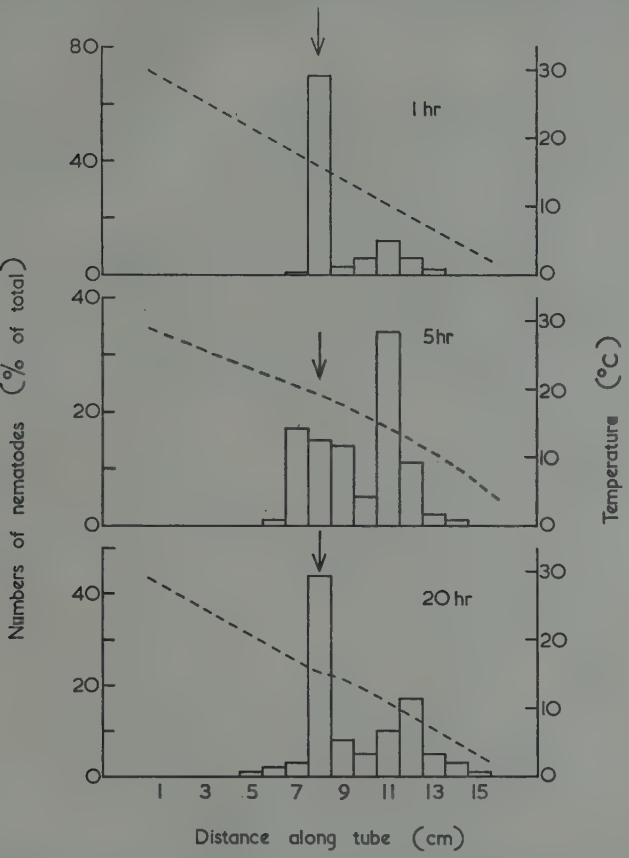

Fig. 3. Distribution of *D. dipsaci* in a 15 cm tube of saturated sand after 1, 5 and 20 hours in a temperature gradient. The arrows show the point of inoculation.

experiment the tube was cut into 1 cm sections and the nematodes were counted. The mobility of *D. dipsaci* in sand at different constant suctions and moisture contents was determined by a method described by Wallace (1958a).

Results

The movement and aggregation of *D. dipsaci* was determined after 20 hours at four different suction ranges 15-25, 20-30, 25-35 and 30-40 cm of water over the moisture characteristic (Fig. 13). In all four experiments the nematodes moved to the higher moisture content end of the gradient (Fig. 5). There was no evidence of a moisture content "preferendum"; thus in the 25-35 suction range a peak

occurred at 25 % moisture content and in the 30-40 suction range at 12 % moisture content. It is apparent from the data in the 15-25 suction gradient (Fig. 5) that *D. dipsaci* responds to gradients as low as 1 % moisture content over a length of 10 cm. The mechanism of orientation is, therefore, very sensitive. The stages

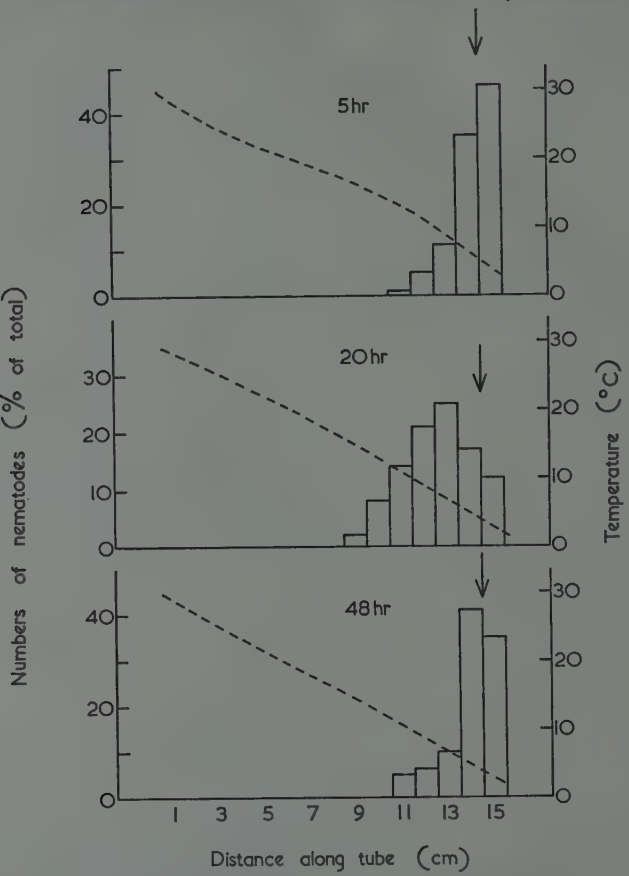

Fig. 4. Distribution of *D. dipsaci* in a 15 cm tube of saturated sand after 5, 20 and 48 hours in a temperature gradient. The arrows show the point of inoculation.

in aggregation with time are shown in Fig. 6. The initial distribution peak at the point of inoculation into the sand is shown at zero time. After 2 hours this peak was reduced and nematodes had moved throughout the tube. After 24 hours there was a distinct aggregation at the high moisture end of the gradient which was even more apparent after 48 hours.

LIGHT

Method

All light experiments were done in a light proof compartment. A light gradient was obtained by passing a vertical beam through an optical wedge on to a horizontal

polythene channel 13 cm long and 6 mm wide, containing 2 per cent agar. A microscope lamp gave a source of light and a cardboard tube was fitted to prevent scatter. A petri dish containing 3 per cent ferric alum, depth 1 cm, was used as a heat filter between lamp and channel (Lees, 1948); measurements with a thermocouple along the agar showed no temperature differences. A gradient of 10

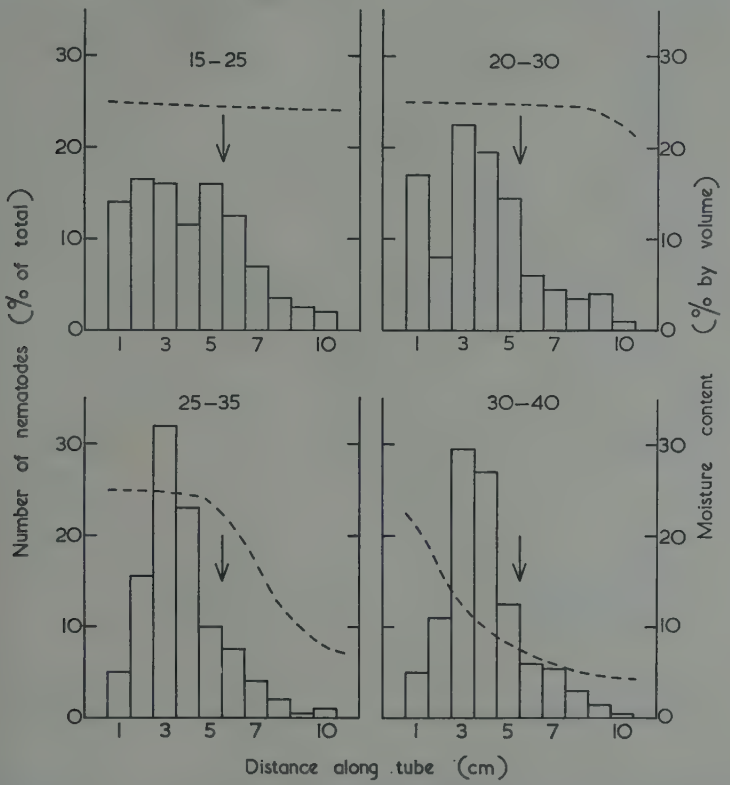

Fig. 5. Distribution of *D. dipsaci* after 20 hours in different gradients of moisture content. The dotted line indicates the moisture content gradient when different suctions were applied. The arrows show the point where the nematodes were inoculated into the sand and the numbers 15-25, 20-30, 25-35 and 30-40 are the suction ranges applied to give the required moisture gradient. Each histogram is the mean of four replicates.

foot candles was obtained over the length of the tube. Light intensity was measured with an S.E.I. exposure photometer. About 100 nematodes were introduced on to the agar at the centre of the channel and, at the end of the experiment, the channel was cut up into 1 cm sections and the nematodes were counted. A similar arrangement was used to study behaviour at a light-dark boundary. Half of the channel was covered and the other half exposed to a constant light intensity of 10 foot candles. Reaction to a source of light was tested by placing the micro-

scope lamp at one end of the channel. Mobility at different constant light intensities from 0 to 46 foot candles was studied by similar means except that the nematodes were introduced at one end of the channel. The nematodes were counted in each cm section along the channel and then by plotting probits of the cumulative percentages against log distance along the tube it was possible to obtain an

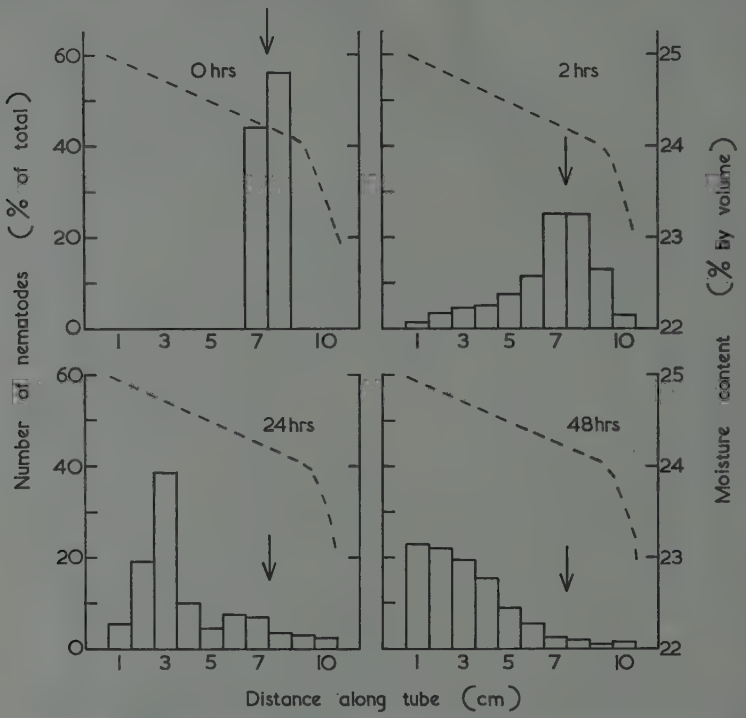

Fig. 6. Distribution of *D. dipsaci* after 0, 2, 24 and 48 hours in the same moisture gradient (dotted line). The arrows indicate the point of inoculation. Each histogram is the mean of four replicates.

objective measure of the distance travelled by 50 per cent of the nematodes. This was used as the criterion of mobility. A heat filter was used in all experiments.

Results

There was no indication of aggregation in a light intensity gradient or orientation to a light-dark boundary or to a source of light. It is concluded that if light has any influence on *D. dipsaci* it is very slight.

PARTICLE SIZE

Method

Seven sizes of sand of mean particle diameter 55, 100, 200, 300, 425, 750, and 1300 μ were used. Polythene tubes 3.5 cm long and 5 mm internal diameter were filled with the different grades in order of increasing size to give a depth of 5

mm for each. The sand was then saturated with water and the tubes placed horizontally. Nematodes were introduced either at the coarse sand end of the tube or through a hole in the tube into the middle of the 300 μ grade sand. At the end of the experiment the tube was cut into seven 5 mm lengths and the nematodes counted in each size of sand. Mobility in the different saturated sand sizes was assessed by introducing nematodes into the end of a horizontal polythene tube

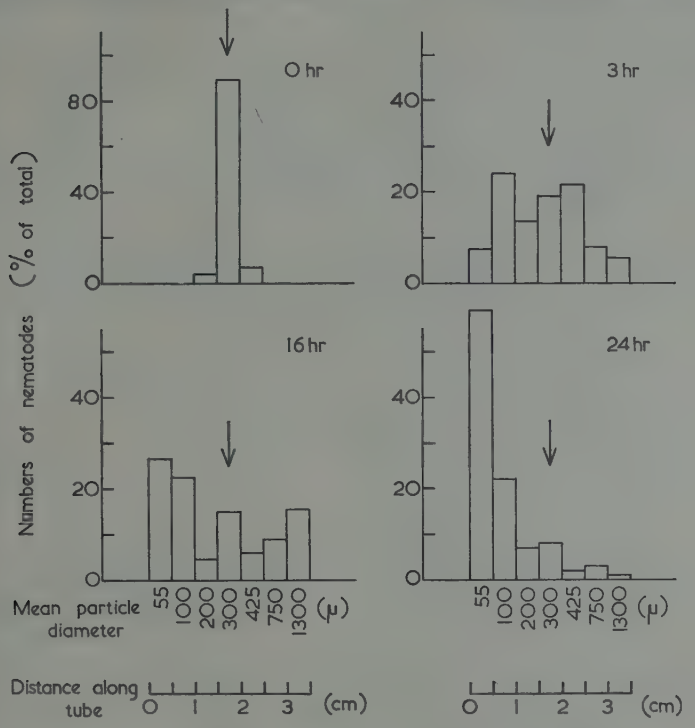

Fig. 7. Distribution of *D. dipsaci* after 0, 3, 16 and 24 hours in a particle size gradient contained in a tube 3.5 cm long. Arrows show the point of inoculation of the nematodes. Each histogram is the mean of four replicates.

(6 cm by 5 mm) containing the sand. After 5 hours the tube was cut up and the percentage of nematodes which had migrated beyond 3 cm was assessed.

Results

When *D. dipsaci* was introduced into the 300 μ particles, the initial peak at zero time was reduced after 3 hours and nematodes were distributed throughout the tube. After 16 hours there was some tendency to aggregate in 55 and 100 μ particles and after 24 hours, this aggregation was definite (Fig. 7). A similar result was obtained if the nematodes were introduced at one end of the tube into 1300 μ sand particles (Fig. 8).

WATER PERCOLATION

Method

The assessment of nematode velocity in horizontal tubes of sand with constant flow of water at different velocities has been described (Wallace, 1959). The term velocity of nematodes refers to the mean velocity of the population introduced into the sand, and populations distributed randomly about the point of inoculation

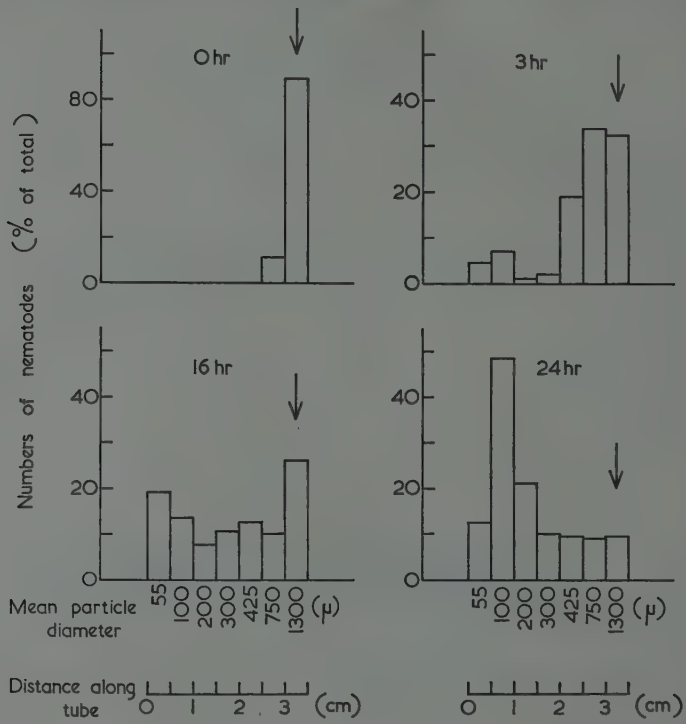

Fig. 8. Distribution of *D. dipsaci* after 0, 3, 16 and 24 hours in a particle size gradient. Arrows show the point of inoculation of the nematodes. Each histogram is the mean of four replicates.

in the tubes at the end of the experiment are considered to have no directional velocity.

Results

The influence of percolating water on the movement of nematodes in soil has been studied by Wallace (1959) who showed that nematode velocity was influenced by the percolation velocity, particle size and nematode body length. Fast flow rates of 500 cm/hr and higher were used and nematode movement was passive in the water stream. The question arises whether *D. dipsaci* at slow water flow rates orientates with the flow stream and whether they swim with the current or, like fish, against it. The velocity of *D. dipsaci* in water percolating at low

flow rates (20-200 cm/hr) through sand of different particle sizes was studied. The velocity of *D. dipsaci* (V) in all particle sizes varied directly as the rate of flow of water (R) but the ratio V/R increased with particle diameter (D) (Fig.

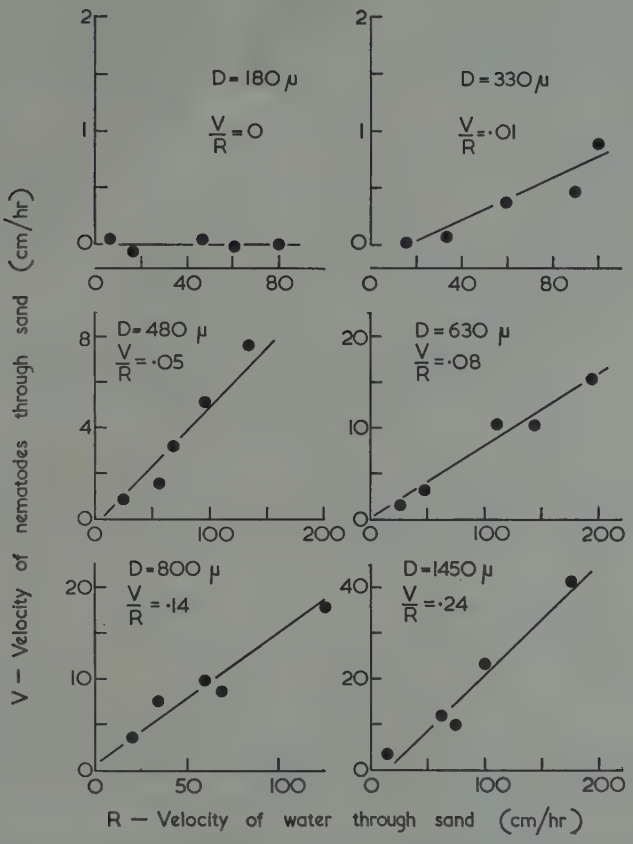

Fig. 9. The relationship between velocity of *D. dipsaci* (V) and the rate of flow of water (R) through horizontal tubes containing sand of six different particle diameter (D). The ratio V/R is given for each particle size.

9). In the 180 μ diameter sand the nematodes moved at random from the point of inoculation and did not show any tendency to move with or against the current although *D. dipsaci* is able to migrate in 180 μ particles (Wallace 1958b). Thus, in sand of this size the water flow has no directional effect on nematode movement. These results show that *D. dipsaci* does not swim against a current of water in sand particles. The relationship between V/R and particle size (D) is a straight line (Fig. 10) and suggests that any directional movements in a current of water are purely passive. These results, however, do not exclude the possibility that the

nematodes are orientated with the water stream or that they swim with the current with their heads pointing downstream. To examine these possibilities large numbers of *D. dipsaci* were observed in a stream of water in a horizontal glass tube. The flow rate was adjusted to about 100 cm/hr and the orientation of nematodes in the stream was noted. The nematodes moved at all angles to the flow and revolved frequently especially when they touched the sides of the tube. A similar

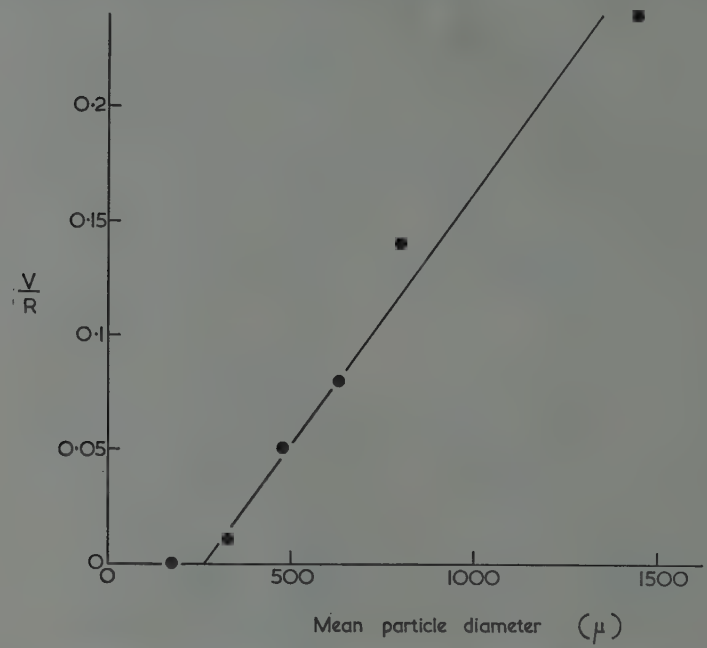

Fig. 10. The relationship between the ratio of nematode velocity and velocity of water flow (V/R) and mean particle diameter in horizontal tubes.

picture was obtained from observations on the movement of *D. dipsaci* in water flowing through sand. Thus, there is no evidence of orientation to a stream of water.

GRAVITY

Method

The technique to determine the downward velocity of nematodes in vertical polythene tubes containing saturated sand of different particle sizes was similar to that used in the study of water percolation.

Results

The influence of gravity on the orientation of nematodes is dependent on the centre of gravity of the nematode. Thus, vinegar eelworm, *Turbatrix aceti*, is tail

heavy and in a liquid medium it swims to the surface with its head uppermost (Peters, 1952). The influence of gravity on nematodes moving in thin films is probably negligible compared with the surface tension forces of the water film but, in water-filled soil pores, gravity may have some effect. The downward velocity of *D. dipsaci* in water-filled pores of sand of different particle diameter varied directly as the particle size but in particles of less than 250 μ diameter the nematodes moved upwards and downwards to the same extent, thus the down-

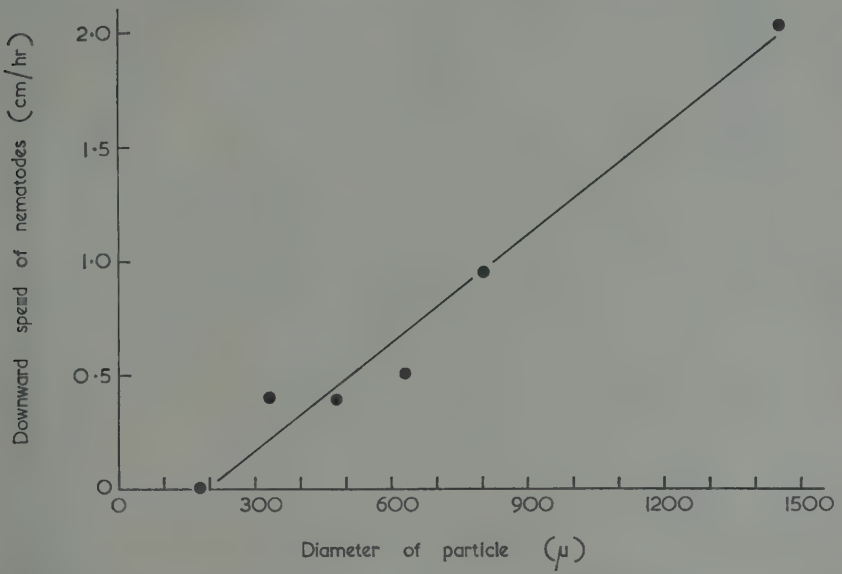

Fig. 11. The influence of particle size on the downward velocity of *D. dipsaci* in vertical tubes containing saturated sand. The water is static.

ward velocity of the population was zero (Fig. 11). The results indicate that there is no tendency to swim upwards and that increased downward velocity with increased particle size is a passive, falling effect. Observations of dead *D. dipsaci* in static water showed that they fell head downwards and that living *D. dipsaci* tended to swim downwards. This suggests that this species is head heavy and that downward movement might be assisted by this orientation. In fact, direct observation of active *D. dipsaci* moving down through 1300 μ sand revealed no indication of orientation.

DISCUSSION

The simplest mechanism of aggregation is an orthokinesis which has been defined by Ewer & Bursell (1950) as variation in generalised, undirected, random locomotory activity caused by variations in the intensity of stimulation. The aggregation of *D. dipsaci* in a temperature gradient cannot be explained in such terms.

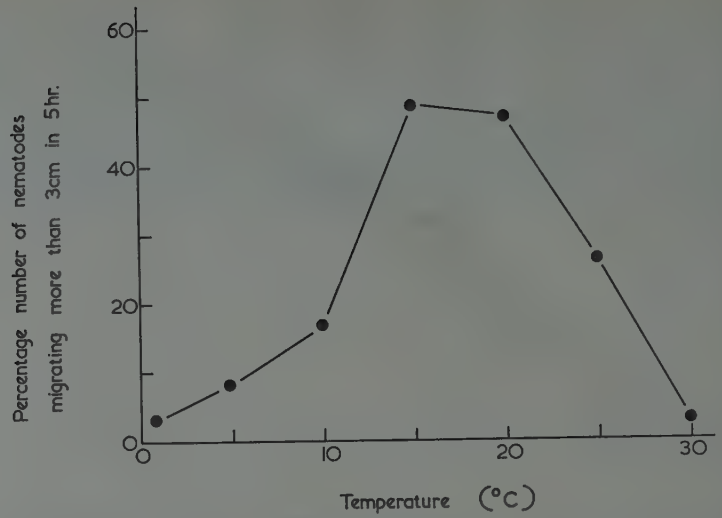

Fig. 12. The mobility of *D. dipsaci* at different constant temperatures. Each point is the mean of five replicates.

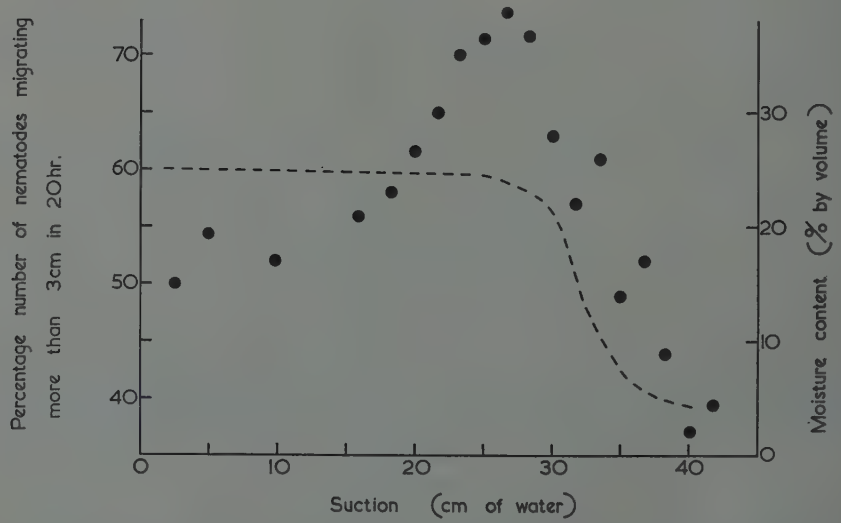

Fig. 13. The mobility of *D. dipsaci* at different constant suctions and moisture content. The dotted line shows the relationship between suction and moisture content (moisture characteristic).

Thus, nematodes were introduced into the gradient at 15° C, the optimum temperature for locomotion (Fig. 12). With a simple speed orthokinesis aggregation would occur at both ends of the gradient where mobility was low. In fact, there was a generalised movement to the cold end with aggregation at about 10° C

(Fig. 3). Some behavioural response to temperature, such as differences in adaptation to a rising or a falling temperature in a gradient, may provide the solution to this problem (Fraenkel & Gunn, 1940) but further work is needed on this aspect.

In moisture gradients *D. dipsaci* aggregates at the end with highest moisture content. The nematodes do not show a generalised movement up the gradient with time as in a temperature gradient, but they spread throughout the whole gradient at first and only after 24 hours is the aggregation well defined (Fig. 6). The

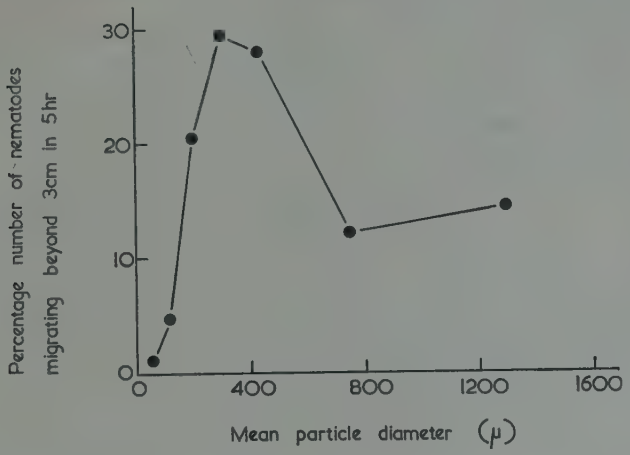

Fig. 14. The mobility of *D. dipsaci* in different particle sizes of saturated sand. Each point is the mean of five replicates.

nematodes in this experiment were introduced into the sand at the optimum moisture content for movement (Fig. 13). Thus with aggregation by a speed orthokinesis nematodes would aggregate at both ends of the gradient where mobility was low. That they aggregate at one end suggests again that some behaviour mechanism is working.

Movement in particle size gradients resembles that in moisture gradients. The nematodes occur throughout the gradient after 3 hours and well defined aggregation occurs only after 24 hours (Figs. 7 and 8). As in previous experiments the nematodes were introduced into the gradient at the optimum conditions for movement (Fig. 14). Aggregation at one end of the gradient in the fine sand is probably a simple orthokinesis because, in the 55 μ sand, activity (wave frequency along the body) and mobility (speed through the sand) are almost zero. In the 1300 μ sand, although mobility is low, activity is high, consequently the nematodes continuously leave and enter this part of the gradient.

In a recent review (Wallace, 1961) the absence of critical techniques in studies on nematode orientation was stressed. There are plenty of data on nematode orientation, chiefly in zoo-parasitic species, but inadequate experimental technique and

absence of controls have invalidated many of the conclusions. Thus, Crofton (1954) has criticised work on geotaxis and has described vertical movements of strongyloid larvae as normal random movement. Parker & Haley (1960) have shown that, contrary to previous conclusions, larvae of *Nippostrongylus muris* are not phototactic but movement to a light source does occur if heat is not carefully excluded. Light, gravity and water percolation do not affect the orientation of *D. dipsaci* although passive directional movement may occur with the last two stimuli. There is evidence, however, that temperature, moisture content and particle size do induce aggregation.

The ecological significance of these results is obvious. In particular, the results may help to explain how *D. dipsaci* is able to live and invade the host plant at the soil surface. Work on similar lines may also help to elucidate behavioural and distributional differences between species in a population and of particular species in different environments.

ZUSAMMENFASSUNG

Die Orientierung von Ditylenchus dipsaci *nach physikalischen Reizen*

Bei einem Temperatur-Gradienten von 2-30° C sammelt sich *D. dipsaci* bei etwa 10° C. Bei einem entsprechenden Gefälle der Feuchtigkeit sammeln sich die Nematoden an der Stelle des höheren Feuchtigkeitsgehalts. Diese Erscheinung kann in beiden Fällen nicht einfach mit einer lokomotorischen Aktivität bei verschiedenem Gefälle erklärt werden, sondern ist wahrscheinlich auf eine verhaltensmässige Reaktion zum Gradienten zurückzuführen.

Bei einem Gefälle der Teilchengrössen sammelt sich *D. dipsaci* in den feineren Teilchen an einem Ende des Gradienten an. Diese Reaktion ist vermutlich eine einfache Orthokinese, weil Bewegung und Aktivität in dem feinen Sand fast Null sind und die Nematoden hier bleiben werden.

Licht, Gewicht und Wasserstrom beeinflussen nicht die Orientierung von *D. dipsaci,* obwohl eine passive gerichtete Bewegung bei den beiden letzten Reizen auftreten kann.

REFERENCES

Crofton, H. D. (1954). The vertical migration of infective larvae of strongyloid nematodes. *J. Helm.* **28**, 35-52.

Ewer, D. W. & Bursell, E. (1950). A note on the classification of elementary behaviour patterns. *Behaviour* **3**, 40-47.

Fraenkel, G. S. & Gunn, D. L. (1940). *The orientation of animals.* vi + 352 pp. Oxford: Clarendon Press.

Lees, A. D. (1948). The sensory physiology of the sheep tick *Ixodes ricinus* L. *J. exp. Biol.* **25**, 145-207.

Parker, J. C. & Haley, A. J. (1960). Phototactic and thermotactic responses of the filariform larvae of the rat nematode *Nippostrongylus muris*. *Exp. Parasit.* **9**, 92-97.

Peters, B. G. (1952). Toxicity tests with vinegar eelworm. I. Counting and culturing. *J. Helm.* **26**, 97-110.

Wallace, H. R. (1958a). Movement of eelworms I. The influence of pore size and moisture content of the soil on the migration of larvae of the beet eelworm, *Heterodera schachtii* Schmidt. *Ann. app. Biol.* **46**, 74-85.

—— (1958b). Movement of eelworms II. A comparative study of the movement in soil of *Heterodera schachtii* Schmidt and of *Ditylenchus dipsaci* (Kuhn) Filipjev, *Ann. appl. Biol.* **46**, 86-94.

—— (1959). Movement of eelworms IV. The influence of water percolation. *Ann. appl. Biol.* **47**, 131-139.

—— (1960). Movement of eelworms VI. The influences of soil type, moisture gradients and host plant roots on the migration of the potato-root eelworm *Heterodera rostochiensis* Wollenweber. *Ann. appl. Biol.* **48**, 107-120.

—— (1961). The bionomics of the free-living stages of zoo-parasitic and phyto-parasitic nematodes — a critical survey. *Helm. Abs.* **30**, 1-22.

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Nematologica 6 (1961): 237-257. Leiden, E. J. Brill

A REDESCRIPTION OF *LONGIDORUS ELONGATUS* (DE MAN, 1876) THORNE & SWANGER, 1936, (NEMATODA, DORYLAIMIDAE) AND DESCRIPTIONS OF FIVE NEW SPECIES OF *LONGIDORUS* FROM GREAT BRITAIN

BY

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Longidorus elongatus (de Man, 1876) Thorne & Swanger, 1936 is redescribed and figured. Five new species: — *L. leptocephalus* n. sp., *L. attenuatus* n. sp., *L. goodeyi* n. sp., *L. caespiticola* n. sp. and *L. macrosoma* n. sp. are described from various British populations. Measurements of the larval stages are given for each species, together with illustrations of their tails.

During examination of soils six distinct species of *Longidorus* were found; one was *Longidorus elongatus* (de Man, 1876) Thorne & Swanger, 1936. Two species very close to *L. elongatus* are described as *L. leptocephalus* n. sp. and *L. attenuatus* n. sp. T. Goodey, (1951), in defining *L. elongatus,* included measurements and figures of a single female from turf at Winches Farm, St. Albans. This specimen is identical with those recently found in two soils and is described as *L. goodeyi* n. sp. A fifth species, close to *L. goodeyi*, is described as *L. caespiticola* n. sp. Another new species, the largest so far found in Britain, is named *L. macrosoma* n. sp.

The *Longidorus* spp. were extracted from turf or soil by sieving. Living and preserved specimens were observed and measurements and drawings were made from British specimens relaxed by heat, fixed in TAF and mounted in glycerin.

Longidorus elongatus: Ten populations of *L. elongatus* were found and compared with topotypes collected by Mr. P. A. A. Loof who says (*in litt.*) that the types of 1876 no longer exist in de Man's collection, although there are specimens collected later. The following description of *L. elongatus* is based on these topotypes.

LONGIDORUS ELONGATUS (DE MAN, 1876) THORNE & SWANGER, 1936

syn. *Dorylaimus elongatus* de Man, 1876
D. (*Longidorus*) *elongatus* de Man, 1876 (Micol., 1922)
Trichodorus elongatus (de Man, 1876) Filipjev, 1921
D. tenuis Linstow, 1879 syn. by de Man, 1884
nec. *L. elongatus* T. Goodey, 1951 see his Fig. 161.

Neotype ♀ L = 5.16 mm; a = 95; b = 19.2; c = 91; V = 50.
Topotypes ♀ L = 4.64 mm; a = 87; b = 11.5; c = 107; V = 47.
♀ L = 3.98 mm; a = 73; b = 11.0; c = 91; V = 49.
(This specimen still within fourth stage larval cuticle.)

The above measurements were made by Mr. Loof from freshly relaxed specimens associated with grass roots in a garden at Havendijk 31, Middelburg, the Netherlands.

When fixed in F.A.A., processed through an alcohol glycerin mixture and mounted in glycerin the specimens had the following measurements: —
Neotype ♀ L = 4.87 mm; a = 122; b = 18.7; c = 128; V = 51; stylet 95 μ. Oral aperture to guide ring 29 μ; tail length 38 μ.
Topotypes ♀ L = 4.85 mm; a = 88; b = 13.5; c = 115; V = 49; stylet 94 μ.
♀ L = 3.75 mm; a = 68; b = 8.5; c = 94; V = 51; stylet 88 μ.
Neotype, slide 168/1/1. Topotype, slides Nos. 168/1/2-3, Nematology Department, Rothamsted. Measurements from de Man, 1876. L = 5.3-5.6 mm; a = 98-100; b = 14-16; c = 100-110; V = 46-50. Calculated from de Man's drawings: — stylet = 86 μ, oral aperture to guide ring 32 μ; tail length 41 μ.

Female. Body forming open 'c' when relaxed by heat, attenuated, gradually tapering towards head, which is half to two-thirds body-width at the guide ring. Lip region, flattened anteriorly, outer ring of ten papillae directed away from oral aperture. Amphid apertures behind lateral lips, very small. Amphids large, pouchlike, almost encircling head and containing coiled nerve organs; they extend half the distance from the oral aperture to guide ring and the base of each is more or less bilobed (Fig. 1, B). Nerve fibres run back from the amphids to sensillae posterior to guide ring. Nerve ring just posterior to the end of the stylet extension. Stylet 95 μ long and slender, with an extension about half its length. Extension consists of triradiate thickenings within oesophageal wall, the thickenings being thickest posteriorly where they tend to open outwards (Fig. 1, A). Walls of oesophagus slightly swollen at, and just behind, base of stylet extension. Guide ring single, prominent, about 30 μ from oral aperture. There is a suggestion of a second guide ring behind the first which appears to be formed by a folding of the stoma lining. This has also been seen in a few specimens from Britain. Anterior part of oesophagus a slender tube which may be coiled or looped before joining the wider, posterior, muscular part; the latter being five times as long as wide and about a third of total length of the oesophagus. The openings of the oesophageal glands occur at the following levels of the posterior part of the oesophagus: — dorsal 15 %, first pair subventrals 59 % and second pair 85 % of its length. Oesophago-intestinal valve bluntly, convex conoid. Pre-rectum well defined about ten anal-body-widths long. Rectum just less than anal-body-width. Anus a short transverse slit. Tail dorsally convex, roundly conoid, as long or slightly longer than anal-body-width with two or three pores on each side.

Hypodermal, lateral cords present, occupying about one quarter of mid-body

Fig. 1. *L. elongatus.* A, topotype, oesophageal region, dorso-lateral; B, neotype, head-lateral; C and D, ♀, from Glendevon, Scotland, C, head-lateral; D, head-ventral; E, ♀, gonads lateral; F, lip region *en face*; G, spicules and lateral guiding piece-lateral; H, vulva-ventral; I, vagina-ventral; J-M, larval tails from Glendevon population — lateral; J, L_1; K, L_2; L, L_3; M, L_4; N, ♂, Elgin, Scotland, tail-lateral; O, neotype, ♀, tail-lateral; P, topotype, ♀ tail-lateral; Q, Glendevon, ♀, tail-lateral.

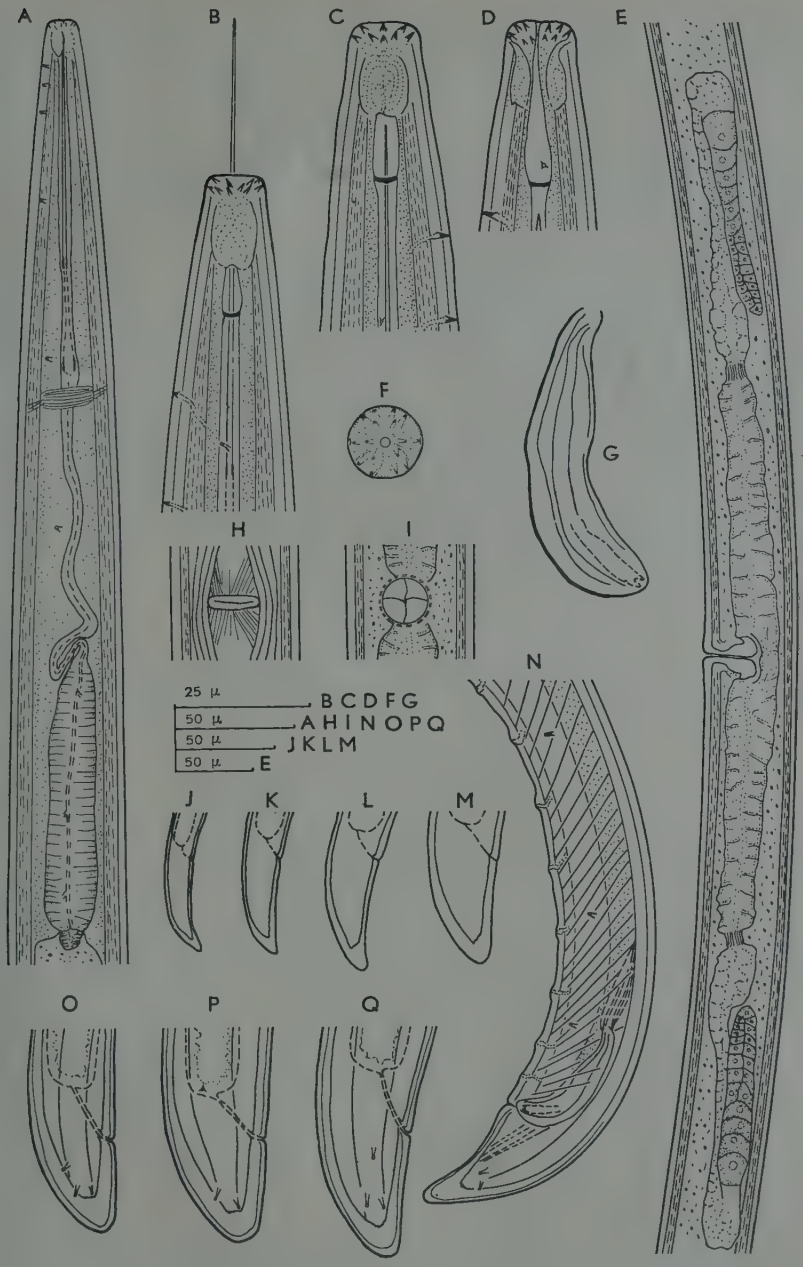

Fig. 1. Legend on foregoing page.

width, glandular in appearance and giving rise to small pores throughout the length of the body which are arranged in a double row, sometimes staggered, at intervals of half to one mid-body-width. The pores are mostly easily seen in the tail and the oesophageal region. The cords narrow towards the oesophageal region and become a thin line with serially arranged pores. There are also four to six ventral pores in the anterior oesophageal region. No dorsal pores seen.

There are thick longitudinal hypodermal muscle bands between the lateral cords. Anteriorly they converge obliquely on to the thin dorsal and ventral cords.

The cuticle is plain apart from pores. In the tail region of some specimens the cuticle is made up of three subsidary layers. The subcuticle has fine transverse striae about 0.5 μ apart, which have a radial appearance at the tail tip. The subcuticle is thicker than the cuticle in the tail region and just behind the lip region.

Gonads paired, opposed, reflexed; reflexed part mainly consists of the ovary in which the larger öocytes are in a single row, the rest multiple (Fig. 1, E). Oviduct and uterus of about equal length, separated by a sphincter like constriction, oviduct with much convoluted walls and the uterus with thick muscular walls. Vulva a transverse slit with a thickened lip about a third of the mid-body-width wide surrounded by four radial muscle bands (Fig. 1, H). Vagina thick walled with + shaped lumen, encircled by a thick muscle band (Fig. 1, I).

No males found at the type locality.

Longidorus elongatus from Britain. Measurements are given in Table I. The lip region in all British specimens is slightly rounder than those from the Netherlands (Fig. 1, C, D), and there is also slight difference in female tail shape (Fig. 1, Q).

Except for this the general appearance and the measurements, including the stylet length, agree closely with the topotypes. A dorsal pore was seen on a few specimens about 20 μ posterior to the guide ring position but was not a constant character.

Males. Only five males were found among more than 150 females and many larvae. Table I gives the measurements and the populations in which they occurred.

Body curled ventrally when relaxed by heat, posterior third more so than the rest. Spicules paired, separate, blunt, arcuate, 58 μ long. Lateral guiding pieces present with furcate distal ends (Fig. 1, G). Tail conoid to blunt conoid, dorsally convex, ventrally concave, with two or three pores on each side — the arrangement varying on different specimens. An adanal pair of supplements present and a median, ventral series of seven to ten. A ventro-lateral series of three to five pores present within the range of the supplements. Oblique copulatory muscles also present in this region (Fig. 1, N). Testes paired, opposed, anterior testis reaching almost to middle of body.

In all specimens examined, both male and female, the amphid pouches extended only about half the distance from the oral aperture to the guide ring.

Larvae. Table II gives measurements of the four larval stages from the Glendevon

Table I

Longidorus elongatus: *Measurements of various populations from Great Britain*

Population			n	Length in mm	a	b	c	V%	Oral aperture to guide ring μ	Stylet length μ	Stylet ext. length μ
Blairgowrie Scot.		♀	6	5.8 (5.0-6.4)	91 (76-109)	12.8 (10.0-14.0)	97 (87-104)	48 (46-51)	34 (32-36)	96 (92-99)	43 (35-49)
Coupar Angus	„	♀	6	5.9 (5.4-6.3)	92 (78-112)	13.1 (11.7-15.7)	97 (78-121)	7^{48}_{7} (45-49)	33 (32-35)	97 (94-101)	44 (39-51)
Elgin	„	♀	17	5.3 (4.8-5.9)	95 (86-108)	12.4 (9.9-14.6)	95 (84-116)	9^{49}_{8} ($6^{-12}45^{-52}5^{-12}$)	33 (31-34)	93 (90-97)	59 (46-66)
		♂	1	5.0	108	12.8	135		30	72	45
Glendevon	„	♀	9	5.6 (4.9-6.0)	95 (79-104)	13.3 (12.1-15.9)	97 (78-106)	8^{49}_{8} ($6^{-11}47^{-52}6^{-10}$)	33 (31-35)	94 (89-100)	58 (56-61)
		♂	1	5.3	113	11.8	113		33	95	58
Grange of Barry	„	♀	6	5.5 (5.0-5.9)	80 (76-89)	13.6 (11.7-15.1)	100 (90-106)	48 (47-51)	33 (32-34)	95 (92-96)	42 (39-44)
Invergowrie	„	♀	12	5.4 (4.6-6.0)	95 (82-111)	13.1 (10.8-14.4)	103 (82-126)	9^{50}_{8} ($7^{-10}46^{-52}7^{-11}$)	34 (33-36)	94 (88-100)	48 (34-61)
St. Cyrus	„	♀	6	5.4 (4.7-6.0)	91 (82-106)	12.6 (11.8-13.8)	92 (73-106)	10^{49}_{10} (47-51)	33 (30-34)	96 (94-97)	39 (38-40)
Woburn, England		♀	3	5.7 (5.5-5.9)	91 (88-97)	16.5 (16.1-17.1)	114 (102-124)	48 (47-49)	35 (34-35)	99 (97-102)	45 (41-48)
Fareham	„	♀	11	5.3 (4.5-6.0)	94 (76-123)	12.9 (9.7-17.5)	101 (80-141)	52 (49-53)	34 (31-35)	90 (83-94)	68 (55-71)
		♂	1	5.3	93	13.0	89		32	94	50
Sunninghill	„	♀	8	5.2 (4.8-5.9)	97 (86-112)	13.0 (11.0-15.4)	98 (75-125)	8^{46}_{8} ($5^{-10}45^{-49}6^{-10}$)	31 (30-33)	88 (81-91)	49 (38-68)

population. Tail shapes are shown in Fig. 1, J-M. The tail length varies little from the first to fourth stage larva, usually increasing only in width. Further observations on page 255 are made on larvae of *Longidorus* spp.

TABLE II

Longidorus elongatus — *Measurements of larvae from Glendevon, Scotland*

Stage	n	L mm	a	b	c	Stylet μ	Stylet Extension μ	Developing Stylet μ
L_1	6	1.1	48	4.9	28	55	35	65
		(1.2-1.4)	(41-54)	(4.1-6.2)	(25-34)	(52-60)	(31-40)	(62-68)
L_2	16	1.8	56	6.2	37	63	43	73
		(1.5-2.1)	(47-63)	(5.3-8.4)	(27-43)	(59-67)	(34-49)	(70-77)
L_3	12	2.7	67	8.1	51	73	50	83
		(2.5-3.2)	(51-85)	(6.5-11.1)	(38-56)	(69-76)	(45-56)	(79-86)
L_4	5	3.9	69	11.6	66	83	55	92
		(3.7-4.1)	(62-81)	(10.6-12.7)	(52-81)	(80-86)	(51-60)	(90-95)

Bionomics. The Scottish populations and the one from Fareham, England were from soil which had been growing strawberries or raspberries. The Sunninghill and Woburn populations came from grass. In all these localities the soil is light to sandy.

Discussion. The specimens collected from the type locality and described above agree closely with the original description by de Man (1876), both in measurements and general appearance. The only difference of note is that de Man figured the lip region as being somewhat expanded whereas those recently collected have an almost continuous lip region. Loof, *in litt.*, says that on slides in de Man's collection there are specimens labelled *elongatus* without an expanded lip region. In some specimens the lip region appears more offset in a dorso ventral view than in a lateral one because of slight contraction by the amphid pouches; this may be the explanation for the slightly expanded lip region shown by de Man.

Thorne (1939) in describing *L. elongatus,* used specimens from Turkey and U.S.A. At least two species are probably represented by Thorne. His illustrated female from Turkey, Fig. 139 and 139c, is probably *L. elongatus.* However, the head of the male from South Carolina, Fig. 139, seems too wide and the amphid pouches too long for *L. elongatus.*

Longidorus sylphus Thorne, 1939 was separated from *L. elongatus* by its continuous lip region and the absence of males. From illustrations of Thorne (1939) *L. sylphus* differs from *L. elongatus* as defined above by its narrower head, shorter distance from the oral aperture to the guide ring and more pointedly conoid tail.

LONGIDORUS LEPTOCEPHALUS N. SP. (Fig. 2, C, D, F, H-M)

Holotype ♀ L = 4.1 mm; a = 96; b = 12.4; c = 96; V = $^{855}7$.

Stylet 63 μ; stylet extension 43 μ; Oral aperture to guide ring 31 μ.

Dimensions of paratypes in Table III.

Female. Body open "c" shape when relaxed by heat. General morphology similar to *L. elongatus.* Lip region narrow, only just over half body-width at the guide ring. Lips rounded, slightly offset with usual number of papillae (Fig. 2, C, F). Amphids large, pouchlike extending half distance from the oral opening to guide ring. Base of each amphid split into two lobes. Oesophagus typical for genus. Oesophageal intestinal valve roundly conoid. Ovaries paired, opposed and reflexed as in *L. elongatus.* Tail dorsally convex, roundly conoid but more pointed than

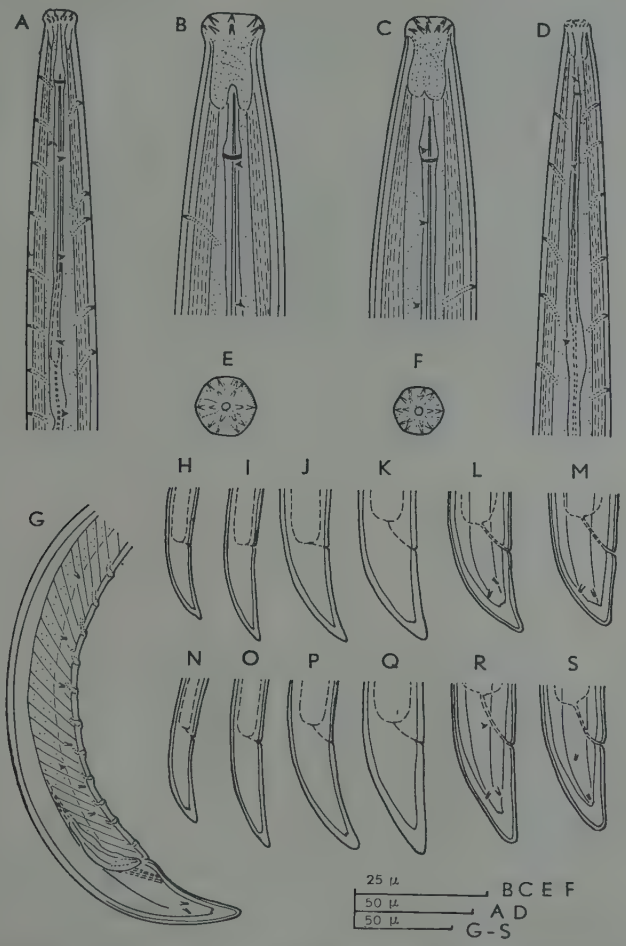

Fig. 2. *L. attenuatus* n. sp. A, anterior oesophageal region-ventral; B, head-lateral E, lip region *en face*; G, ♂ tail-lateral; N-Q larval tails-lateral; N, L_1; O, L_2; P, L_3; Q, L_4; R and S, ♀ tails lateral.

L. leptocephalus n. sp. C, head-lateral; D, anterior oesophageal region-ventral; F, lip region *en face*; H-K, larval tails-lateral, H, L_1; I, L_2; J, L_3; K, L_4; L and M, ♀ tails lateral.

in *L. elongatus,* about one and a quarter anal-body-widths long. A pair of papillae present on each side of the tail (Fig. 2, L, M). Lateral cords about half mid-body-width narrowing towards head, becoming a thin line in the oesophageal region with serially arranged pores. There are three to five ventral pores and a single dorsal pore which is situated about 25 μ posterior to the guide ring. Pre-rectum usually well-defined and about ten anal-body-widths long.

TABLE III

Longidorus leptocephalus *n. sp. Measurements of larval stages and adults*

					♀	♀
Larval stage	L_1	L_2	L_3	L_4	Highfield	Sunninghill
n	9	15	3	8	16	4
Length mm	1.1	1.6	2.3	3.3	4.2	4.2
	(0.9-1.2)	(1.3-1.8)	(2.1-2.4)	(2.9-3.7)	(3.5-4.9)	(3.9-4.6)
a	61	65	82	90	96	96
	(53-68)	(49-81)	(79-87)	(82-114)	(78-127)	(83-121)
b	6.4	7.1	8.9	10.6	13.3	12.0
	(5.1-7.4)	(5.9-9.2)	(7.9-10.2)	(7.9-11.7)	(11.1-20.0)	(11.7-12.4)
c	33	43	65	73	94	96
	(27-43)	(31-65)	(58-69)	(53-82)	(75-128)	(78-105)
v	—	—	—	—	$\frac{8}{54}\frac{7}{52}$	$\frac{8}{53}\frac{7}{52}$
					(4-10 52-576-9)	(6-10 52-557-8)
Stylet μ	34	42	50	56	64	64
	(31-36)	(38-45)	(49-52)	(47-61)	(60-70)	(58-68)
Stylet extension	16	24	31	33	46	34
	(13-21)	(21-31)	(28-34)	(27-37)	(41-56)	(30-36)
Developing stylet	42	49	58	65	—	—
	(39-43)	(45-52)	(55-59)	(63-66)	(-)	(-)
Oral aperture to guide ring	—	—	—	—	30	31
					(29-32)	(30-32)

No males were found among over 100 females, and many larvae.

Larvae: — Heads of larval stages similar in shape to those of adults. Larval tails are shown in Fig. 2, H-K and measurements of the larval stages are given in Table III.

Holotype ♀ slide No. 168/3/1, Paratypes, slides Nos. 168/3/2-22, Nematology Department, Rothamsted.

Type locality. Permanent grassland in the eastern side of Highfield, midway along the Geesecroft fence and some 15 to 20 yards west of it, Rothamsted Experimental Station. About 200 adults and larvae were the most obtained from 100 gms of soil.

Also found with *L. elongatus* in turf from an orchard at Ashurst Lodge, Sunninghill, Ascot, Berks.

Diagnosis. L. leptocephalus differs from *L. elongatus* by its narrower head, shorter stylet and more pointed tail. It resembles *L. sylphus* Thorne, 1939 but the stylet is shorter, the lip region is more rounded and the vulva more posterior.

LONGIDORUS ATTENUATUS N. SP. (Fig. 2, A, B, E, G, N-S)

Holotype ♀ L = 6.0 mm; a = 201; b = 18.9; c = 107; V = 8497.
Stylet 75 μ; stylet extension 43 μ; Oral aperture to guide ring 29 μ.

Allotype ♂ L = 6.3 mm; a = 171; b = 17.1; c = 114; stylet 84 μ; stylet extension 38 μ; Oral aperture to guide ring 32 μ.

General morphology similar to *L. elongatus*. Lip region wide, two thirds the body width at the guide ring, flattened anteriorly and markedly expanded. Amphids large, distinctly bilobed, almost encircling head and extending halfway to guide ring (Fig. 2, B). Oesophagus typical for genus; oesophago-intestinal valve roundly conoid. Lateral cords about half mid-body-width, tapering to a thin line in the oesophageal region where lateral pores are serially arranged. The oesophageal region has five to eight ventral pores but no dorsal pore was seen.

Female. Body slender and only slightly curved when relaxed by heat, the posterior half the more so. Gonads paired, opposed, reflexed as in *L. elongatus*. Tail dorsally convex, conoid, one and a half to one and three quarters anal-body-widths long. A pair of caudal papillae present on either side of tail (Fig. 2, R, S).

Male. Only two males were found among more than 100 females. Body slender, posterior half curved upon itself when relaxed by heat. Spicules large, 45 μ long, arcuate, lateral guiding pieces with bifid ends. An adanal pair of supplements present and a ventral series of 10. Tail dorsally convex, ventrally concave, narrowly conoid with two pores on each side. Prominent copulatory muscles are present and there is a ventro-lateral series of pores within the range of the supplements as well as lateral pores (Fig. 2, G).

TABLE IV

Longidorus attenuatus *n. sp. Measurements of larval stages and adults*

Larval stage	L_1	L_2	L_3	L_4	♀	♂
n	7	4	6	8	15	2
Length mm	1.1	1.7	2.7	4.2	6.1	5.9
	(1.0-1.3)	(1.5-1.8)	(2.3-2.8)	(3.8-4.5)	(5.2-7.3)	(5.5-6.3)
a	64	67	80	101	158	135
	(56-78)	(54-72)	(68-95)	(83-120)	(120-210)	(119-171)
b	6.0	6.3	8.3	12.1	15.2	15.1
	(4.8-6.7)	(6.2-6.5)	(5.1-9.5)	(9.9-14.4)	(12.7-18.9)	(12.9-17.1)
c	32	34	47	67	97	107
	(28-39)	(31-35)	(35-60)	(47-76)	(82-131)	(99-114)
v	—	—	—	—	7498	—
					(7-1244-5569)	—
Stylet μ	47	48	60	69	78	82
	(44-49)	(43-52)	(55-64)	(67-71)	(73-84)	(80-84)
Stylet extension μ	24	30	36	40	41	39
	(18-30)	(27-32)	(32-35)	(35-49)	(36-45)	(38-40)
Developing stylet μ	51	62	68	79	—	
	(46-55)	(57-67)	(63-71)	(75-86)	—	
Oral aperture to guide ring μ	—	—	—	—	28	31
					(26-30)	(30-32)

Larvae. Heads of larval stages similar to those of adults. Tails of larval stages are shown in Fig. 2 N-Q and larval measurements are given in Table IV.
Holotype ♀ slide No. 168/4/1, Allotype ♂ slide No. 168/4/2, Paratypes, slides Nos. 168/4/3-16, Nematology Department, Rothamsted Experimental Station.
Type locality. Light sandy soil in which lettuce had been grown. Hellesdon, Norwich, England.
Diagnosis. L. attenuatus is nearest to *L. elongatus* from which it differs by its more slender appearance, greatly expanded lip region, shorter stylet, narrower and more conoid tail in both sexes.

LONGIDORUS GOODEYI N. SP. (Fig. 3)

Syn. *L. elongatus* in T. Goodey, 1951, Fig. 161; from Winches Turf, St. Albans, England.
Holotype ♀ L = 6.2 mm; a = 80; b = 12.4; c = 128; V = $^{9}53^{9}$.
stylet 97 μ, Oral aperture to guide ring 33 μ.

Dimensions of paratypes in Table V.

TABLE V

Longidorus goodeyi *n. sp.* — *Measurements of larval stages and adults*

Larval stage	L_1	L_2	L_3	L_4	♀
n	14	9	25	19	27
Length mm	1.5	2.3	3.6	5.1	6.7
	(1.4-2.0)	(2.0-2.6)	(3.0-4.6)	(4.4-6.1)	(5.6-7.7)
a	53	63	70	74	85
	(45-61)	(53-76)	(52-87)	(60-87)	(67-117)
b	6.5	8.1	10.4	11.9	14.8
	(4.9-9.8)	(6.6-9.7)	(8.2-13.2)	(9.0-15.7)	(10.1-23.0)
c	31	65	79	96	133
	(27-37)	(48-72)	(57-95)	(75-130)	(99-154)
V	—	—	—	—	$^{115}10.5$ $^{7\text{-}14}50\text{-}55^{9\text{-}13}$
Stylet μ	59	64	77	87	101
	(57-61)	(61-67)	(65-83)	(80-91)	(96-109)
Stylet extension μ	43	44	47	47	61
	(37-50)	(36-53)	(37-55)	(34-72)	(42-79)
Developing stylet μ	64	76	87	100	—
	(61-69)	(70-80)	(80-101)	(95-107)	—
Oral aperture to guide ring μ	—	—	—	—	35 (30-40)

Female. Body takes the shape of a closed "c" or flat spiral when relaxed by gentle heat; more robust in appearance than *L. elongatus* but general morphology similar. Lip region bluntly rounded, one fifth of mid-body-width wide with six fused lips and usual arrangement of papillae. In face view the lateral papillae appear closer together than in other species herein described (Fig. 3, C). Amphid apertures behind lateral lips appearing as very small oval pores in lateral view, crescentic slits in face view (Fig. 3, C). Amphids large, bilobed pouches almost

encircling the head. Ventral lobe of each amphid twice the length of dorsal lobe and reaching to guide ring. (Fig. 3, B, D).

There is a slight but characteristic narrowing of the body between the guiding ring and lip region. One or two ampullae often visible lying beside the anterior

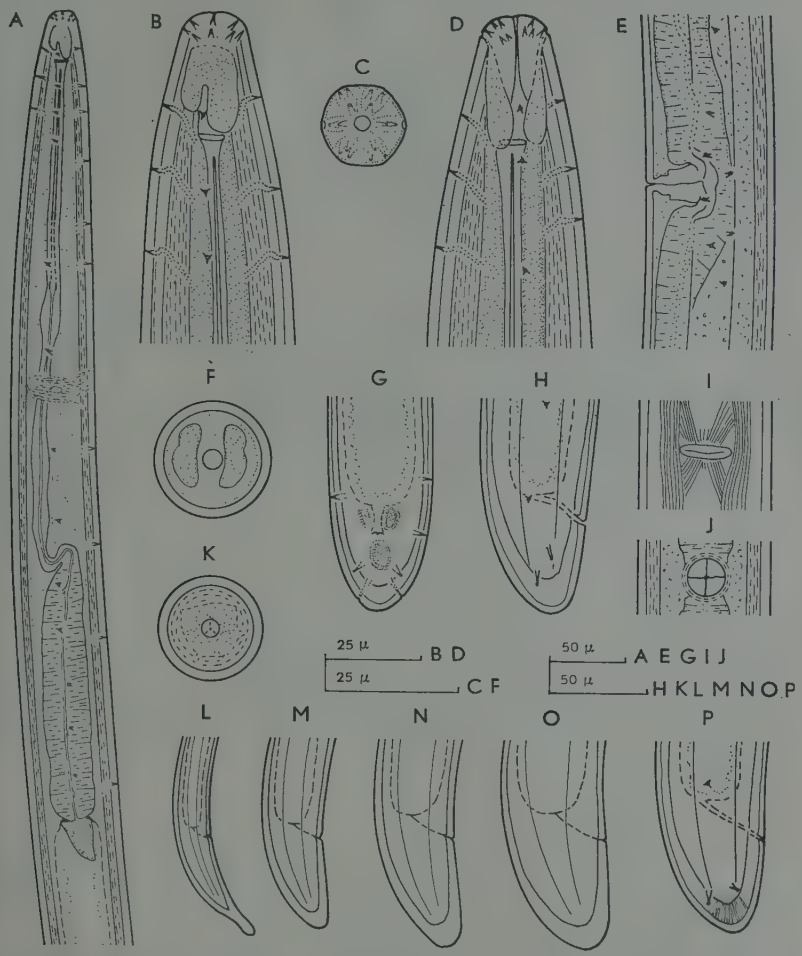

Fig. 3. *L. goodeyi* n. sp. A, Oesophageal region-lateral; B, head-lateral; C, lip region *en face*; D, head-dorsal; E, vulval region-lateral; F, section across amphid pouches near base of ventral lobe; G, ♀, tail-ventral; H and P, ♀, tails-lateral; I, vulva ventral; J, vagina-ventral; K, section across base of stylet extension; L-O larval tails-lateral; L, L_1; M, L_2; N, L_3; O, L_4.

half of the oesophagus. Oesophago-intestinal valve elongate conoid. Prominent sphincter like constriction present between the uterus and oviduct in the paired, opposed, reflexed gonads.

Tail dorsally convex, bluntly conoid, about as long as anal-body-width, with two or three pairs of caudal pores (Figs. 3, H, P). Anus a transverse, bicrescentic slit (Fig. 3, G). Rectum four fifths anal-body-width long. Prerectum usually distinct, six to thirteen anal-body-widths long.

Hypodermal lateral cords one quarter width of mid-body region, relatively wider in anal region. Each narrowing anteriorly to become a thin line in the oesophageal region with serially placed pores. Throughout most of the body length the lateral pores are in a more or less single line spaced at intervals of half to one body-width. In the vulval region the lateral pores are in a double line and more closely spaced. (Fig. 3, E). Ventral pores extend at intervals of half to one body-width from the anterior end for about 20 % of the body-length. There are usually three, occasionally four, dorsal pores present in the oesophageal region (Fig. 3, A).

No males found.

Larvae. Measurements of the four stages are in Table V. Tails of first stage larvae elongate digitate, three anal-body-widths long. Tails of second, third and fourth stages become progressively wider and blunter (Fig. 3, L-O) although the actual length of the tails remains about the same. The heads and amphid arrangement of all the stages seem identical, except for size, to those of the adults.

Holotype ♀ slide No. 168/5/1, Paratypes, slides Nos. 168/5/2-26. Nematology Department, Rothamsted.

Type locality: — Permanent turf on west edge of path beside the Nematology Department museum plots leading from Coach Lane to West-Building, Rothamsted.

Other localities: Turf at Winches Farm, St. Albans. 1 ♀. Collected by T. Goodey. Turf beside garden allotment, type locality for *L. caespiticola* n. sp.

Diagnosis. Longidorus goodeyi is near to but differs from *L. elongatus* in its more robust appearance, different head and tail shape and especially in its larger asymmetrical bilobed, amphids, by the possession of more than one dorsal pore and more ventral pores. The bicrescentic anal opening and the shape of the first stage larval tails are also characters of this species.

LONGIDORUS CAESPITICOLA N. SP. (Fig. 4)

Holotype ♀ L = 7.1 mm; a = 73; b = 12.2; c = 122; V = $^{115}12$; Stylet 111 μ; stylet extension = 59 μ. Oral aperture to guide ring = 35 μ.

Allotype ♂ L = 7.0 mm a = 73; b = 12.1; c = 103; T = 56.
stylet = 111 μ; stylet extension 66 μ; Oral aperture to guide ring = 38 μ.

Measurements of paratypes in Table VI.

General anatomy similar to *L. elongatus* and *L. goodeyi*. Lip region smoothly rounded almost cone shaped, one fifth to one sixth of mid-body-width, bearing the usual arrangement of papillae (Fig. 4, B, C).

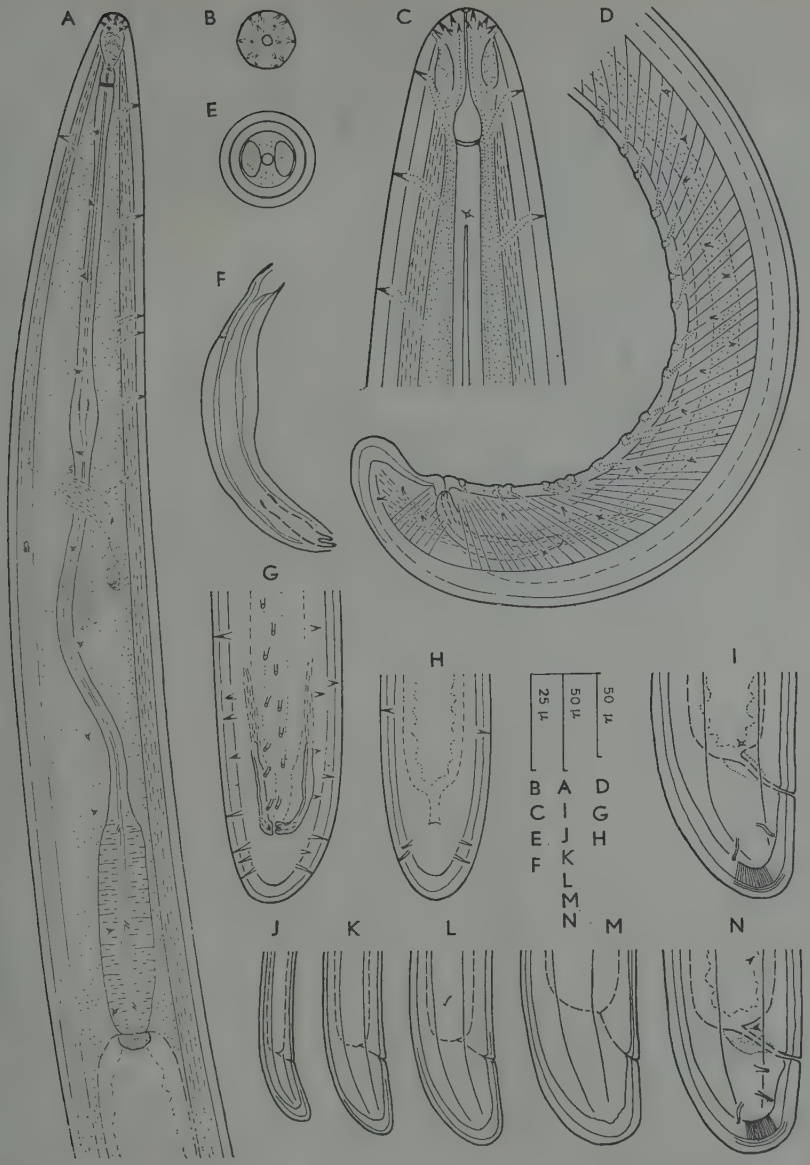

Fig. 4. *L. caespiticola* n. sp. A, oesophageal region-lateral; B, lip region *en face*; C, head-ventral; D, ♂ tail-lateral; E, section across base of amphid lobes; F, spicule and lateral guiding piece-lateral; G, ♂ tail-subventral; H, ♀ tail-ventral; I and N, ♀, tails-lateral; J-M larval tails-lateral, J, L_1; K, L_2; L, L_3; M, L_4.

Amphid openings small, pore-like behind lateral lips. Amphids large and almost encircling head, not lobed. In lateral view they appear to taper posteriorly into the nerve tube but, in dorsal or ventral view, they appear pouch-shaped and extend about halfway to the guide ring (Fig. 4, A, C, E). Hypodermal lateral cords a third to a quarter the mid-body-width with pores at intervals of one body width in a single line, except in the vulval region where the pores are closer together in more of a staggered double line as in *L. goodeyi*. Each cord narrows to a thin line in the oesophageal region, where there are also five or six ventral pores and a single dorsal pore (Fig. 4, A).

TABLE VI

Measurements of Longidorus caespiticola *n. sp.*

Population:	Rothamsted		Bere Ferrers	Essex	Lanivet	Milsted	
	♀	♂	♀	♀	♀	♀	♂
n	16	9	1	1	2	2	2
Length mm	6.7 (5.2-8.2)	6.6 (6.0-7.5)	6.1	6.6	6.3 (5.8-6.7)	6.4 (6.1-6.6)	6.0 (5.9-6.2)
a	72 (64-80)	73 (64-83)	65	66	74 (72-76)	71 (60-82)	64 (62-66)
b	12.6 (10.4-16.2)	12.6 (11.3-15.5)	11.6	11.8	13.2 (10.9-15.5)	11.6 (11.0-12.1)	11.3 (10.6-12.0)
c	103 (83-125)	104 (76-119)	108	133	121 (119-122)	116 (109-123)	122 (119-124)
V or T%	$^{9}53^{9}$ ($^{9\text{-}14}48\text{-}56^{9\text{-}14}$)	56 (54-60)	$^{9}56^{9}$	43	55 (53-56)	$^{12}50^{11}$ ($^{11\text{-}13}48\text{-}51^{10\text{-}12}$)	53 (52-54)
Stylet μ	110 (109-114)	105 (99-111)	117	111	107 (107)	109 (107-111)	111 (104-118)
Stylet extension μ	68 (57-90)	68 (52-78)	74	73	57 (57)	67 (64-70)	67 (65-68)
Oral aperture to guide ring μ	37 (34-41)	39 (32-45)	40	33	41 (38-44)	32 (32)	34 (33-35)

Female. Body forms open "c" when relaxed by heat, the posterior half more curled than the anterior. Gonads paired, opposed, reflexed as in *L. goodeyi*. Tail dorsally convex, bluntly conoid, equal to, or slightly shorter than, anal-body-width and with two or three pairs of caudal pores (Fig. 4, I, N). Rectum two thirds anal-body-width long, pre-rectum usually well defined, eight to thirteen times the anal-body-width. Anus a short transverse slit (Fig. 4, H).

Male. Posterior third of body ventrally curved upon itself when relaxed by heat. Spicules large, 90 μ long, arcuate with bifid lateral guiding pieces (Fig. 4, F). Anus a transverse slit. There is an adanal pair of supplements and a ventral series of 15 to 20 arranged in a staggered, double row (Fig. 4, D, G).

Tail about one anal-body-width long, dorsally convex, ventrally concave, with a bluntly rounded terminus. There are two or three pores on each side of the tail and a ventro-lateral series within the range of the supplements, in addition to

those in the lateral cord (Fig. 4, D). Prominent copulatory muscles present in the range of the supplements.

The ratio of males to females was about 1 : 2.

TABLE VII

Longidorus caespiticola *n. sp. Measurements of larval stages*

Larval stage	n	L mm	a	b	c	Stylet	Stylet ext.	Dev. Stylet
L_1	11	1.3	51	5.3	43	56	38	64
		(1.1-1.3)	(44-56)	(3.9-6.7)	(35-57)	(52-60)	(28-43)	(59-67)
L_2	4	1.8	62	6.6	53	62	42	72
		(1.4-2.4)	(51-65)	(5.3-8.5)	(37-66)	(59-66)	(32-50)	(71-73)
L_3	3	3.0	58	8.7	67	73	55	92
		(2.4-3.4)	(44-73)	(8.3-8.9)	(57-73)	(65-80)	(50-61)	(86-95)
L_4	13	4.5	67	9.4	89	96	64	111
		(4.2-5.8)	(58-78)	(8.5-11.8)	(64-102)	(89-100)	(50-71)	(101-120)

Larvae. Measurements of the four larval stages are in Table VII. Tail of first stage larva twice the anal-body-width, dorsally convex bluntly conoid. The actual tail length increases slightly from first stage larva to the adult but becomes progressively shorter in relation to the anal-body-width (Fig. 4, J-M).

Holotype ♀ slide No. 168/6/1; Allotype ♂ slide No. 168/6/2; Paratypes, slides Nos. 168/6/3-20; Nematology Department, Rothamsted.

Type locality. Turf on west side of wide path which runs north to south between the garden allotments from the "Club House". The site being some 95 yards south of the "Club House". Rothamsted.

L. goodeyi occurs at this site also.

Also found in soil growing Blackberries, Essex; Cherry trees, Milsted, Kent; Strawberries at Lanivet, Cornwall and Fareham, Hampshire. Found also at Bere Ferrers, Devon, and grassland, Tamar Valley, Devon.

Diagnosis. This species is closest to *L. goodeyi* from which it differs in head shape, size and shape of amphids and in possessing a single dorsal pore compared with three or four. Males common. The first stage larvae have bluntly conoid tails whereas those of *L. goodeyi* have digitate tails.

LONGIDORUS MACROSOMA N. SP. (Fig. 5)

Holotype ♀ L = 11.1 mm; a =98; b = 18.3; c = 233; V = $^{105}6^{10}$; Stylet 123 μ; stylet extension 64 μ; Oral aperture to guide ring 42 μ.

Allotype ♂ L = 9.8 mm; a = 86; b = 16.1; c = 201; stylet 132 μ; stylet extension 57 μ; oral aperture to guide ring 40 μ.

For measurements of paratypes see Table VIII.

Body long and robust, 8-11 mm, narrowing towards the lip region which is truncate, flattened to slightly concave, not offset and one quarter to one fifth mid-body-width. Lips fused with the usual arrangement of sixteen papillae directed away from the oral aperture (Fig. 5, C, F).

Table VIII

Measurements of Longidorus macrosoma *n. sp.*

Population		n	L mm	a	b	c	V or T%	Stylet μ	Stylet Extension μ	Oral aperture to guide ring μ
Geesecroft	♀	8	10.5	100	18.6	225	52	134	71	42
			(8.4-11.9)	(89-126)	(17.0-20.3)	(205-253)	(47-56)	(123-140)	(63-92)	(38-45)
	♂	7	10.1	103	16.5	217	—	135	59	43
			(9.2-11.1)	(86-116)	(12.8-19.9)	(194-268)	(-)	(128-139)	(53-66)	(40-46)
East Hyde	♀	5	8.6	93	15.9	200	$^{10}52^{9}$	124	62	40
			(7.9-9.3)	(84-101)	(14.0-20.6)	(187-208)	(48-54)	(119-128)	(53-75)	(40-41)
	♂	3	8.6	104	13.1	202	54	125	67	42
			(8.1-9.1)	(101-109)	(12.5-14.1)	(160-226)	(46-63)	(123-127)	(62-72)	(40-43)
Roundwood Land	♀	11	9.3	93	13.9	176	$^{8}49^{8}$	131	84	43
			(8.4-10.2)	(77-113)	(11.0-16.4)	(148-230)	$(^{4\text{-}10}45\text{-}^{51\,5\text{-}10})$	(124-136)	(75-95)	(41-48)
	♂	5	8.7	88	14.7	202	45	125	81	41
			(7.9-9.7)	(76-96)	(13.0-17.3)	(159-242)	(41-54)	(113-140)	(70-97)	(39-43)
Colchester	♀	15	10.1	109	15.5	229	$^{9}51^{9}$	137	79	42
			(8.5-11.7)	(84-142)	(13.2-21.0)	(164-262)	$(^{6\text{-}11}47\text{-}^{55\,7\text{-}13})$	(122-148)	(69-93)	(37-47)
	♂	3	10.1	101	14.2	218	52	130	75	45
			(9.7-10.6)	(97-106)	(13.1-14.8)	(193-249)	(51-53)	(127-140)	(71-81)	(41-47)
Harwell	♀	1	7.1	79	14.0	179	$^{10}52^{10}$	122	78	39
	♂	1	9.2	76	12.9	229	53	125	78	43

Amphids large, almost encircling the head. In lateral surface view they appear to taper posteriorly and reach as far back as the guide ring. In a deeper lateral

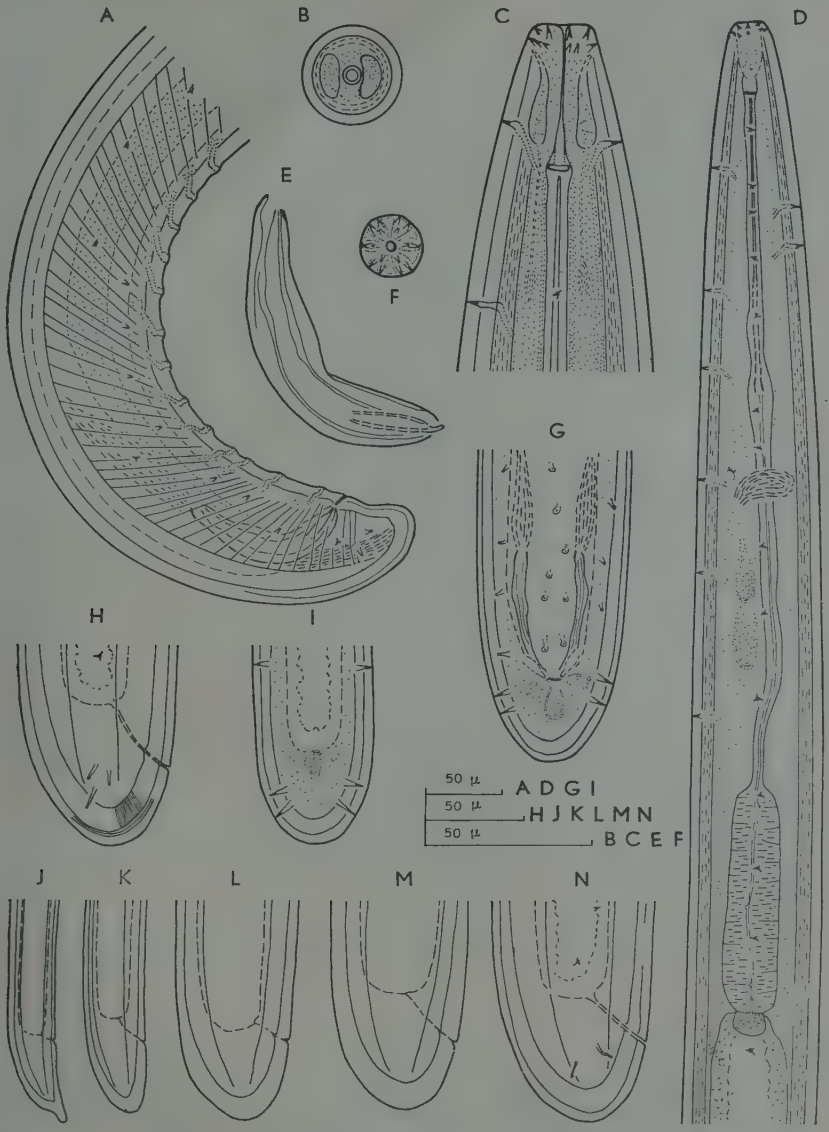

Fig. 5. *L. macrosoma* n. sp. A, ♂ tail-lateral; B, section across amphids 20 μ from oral aperture; C, head-ventral; D, Oesophageal region-lateral; E, spicule and lateral guiding piece-lateral; F, lip region *en face*; G, ♂ tail-ventral; H and N, ♀ tails-lateral; I, ♀ tail ventral; J-M larval tails-lateral; J, L_1; K, L_2; L, L_3; M, L_4.

view they have a bilobed appearance and in a dorso-ventral view they appear pouchlike (Figs. 5, B, C, D). Openings small, slitlike behind the lateral lips. Stylet and oesophagus typical for the genus (Fig. 5, D). One or two ampullae sometimes visible lying beside the anterior part of the oesophagus. Oesophago-intestinal valve varies in shape from a flattened sphere to bluntly conoid. Hypodermal lateral cords occupy about a quarter of the mid-body-width, narrowing anteriorly. The arrangement of the lateral pores varies from a staggered double line to a single line. Two to four dorsal pores and five to eight ventral pores are present in the anterior two thirds of the oesophageal region.

Female. Body forms open "C" shape when relaxed by heat, the posterior half more ventrally curved. Gonads paired, opposed, reflexed as in *L. goodeyi.*

Tail hemispherical to blunt, dorsally convex conoid, short, half to two thirds anal-body-width with a pair of caudal pores on each side and sometimes a third pore opposite the anus (Fig. 5, H, N). Anus a transverse elongate slit about one tenth anal-body-width. Rectum just over half an anal-body-width long. Pre-rectum usually well defined, twenty to thirty times the anal-body-width long.

Male. Posterior third of body curled like a loose spring when relaxed by heat. Testes paired, opposed, equal in length and about half the length of the vas deferens. Spicules large, bluntly arcuate, 105 μ long with lateral guiding pieces the ends of which do not appear to be bifurcate (Fig. 5, E). Cloacal opening short, transverse. Supplements consist of an adanal pair and a staggered ventral series of fifteen to nineteen. Sometimes there are two adanal pairs of supplements within the range of the spicules before they become staggered (Fig. 5, G). Tail dorsally convex, bluntly conoid with a shallow post anal depression. Caudal pores vary from two to four on each side of the tail. The pores continue in two lines anterior to the spicules, but within the range of the supplements there are nearly twice as many in the ventro-lateral series as in the lateral (Fig. 5, A). Anterior to the supplements the lateral pores are arranged as in the female. Prominent copulatory muscles within the range of the supplements.

The ratio of males to females varied from 1 : 1 to 1 : 4.

Larvae. Table IX gives measurements of larval stages of the Roundwood Lane population.

TABLE IX

Longidorus macrosoma *n. sp. Measurements of larval stages*

Larval stage	n	L mm	a	b	c	Stylet length μ	Stylet extension μ	Developing stylet μ
L_1	11	1.8	61	5.8	43	75	51	89
		(1.6-2.2)	(55-73)	(4.6-7.6)	(35-54)	(69-80)	(36-69)	(84-95)
L_2	6	2.9	67	7.3	71	88	54	106
		(2.6-3.3)	(60-75)	(5.8-8.5)	(63-80)	(85-94)	(44-65)	(104-113)
L_3	21	4.7	76	9.5	90	104	62	120
		(3.7-5.5)	(62-98)	(6.2-11.2)	(81-99)	(92-117)	(50-72)	(109-129)
L_4	16	6.7	85	13.1	126	121	66	137
		(5.9-7.8)	(65-96)	(11.5-15.3)	(93-150)	(115-129)	(52-83)	(130-145)

First stage larvae have digitate tails two anal-body-widths long. The tails become progressively rounder from second stage to adults (Fig. 5, J-M). The heads of all the larval stages have the typical truncate appearance of the adults.
Holotype ♀ slide No. 168/7/1, Allotype ♂ slide No. 168/7/2, Paratypes, slides Nos. 168/7/3-82, Nematology Department, Rothamsted.
Type locality. Soil in established woodland in Geesecroft Wilderness; 10 yards in from the road Harpenden — Redbourn, Rothamsted Experimental Station.
Other localities. Soil about the roots of elder bush in a hedgerow on the north side of Roundwood Lane, Harpenden: map reference O.S. 1 in 52/108147. Soil about the roots of elder bush in a hedge where it joins the north side of the Thrales End to East Hyde road, about 300 yds. west of the railway bridge; Harpenden: map reference O.S. 1 in. 52/126169. Also found associated with blackberry roots, Wakes Colne near Colchester, Essex; Cherry soil, Harwell and fallow raspberry soil, Latton, Wilts; in hedgerows or woods at Marsh and Slapton, Bucks; Thame, Oxon. and Tilsworth, Beds. All these localities have medium to heavy textured soil types.
Diagnosis. L. macrosoma is easily recognized because of its large size, robust appearance and truncate lip region. It is closest to *L. maximus* (Bütschli, 1874) Thorne and Swanger, 1936, but differs in having a continuous fused lip region instead of an offset one with distinct lips. Also the tail is shorter and rounder than *L. maximus.*

Observations on Longidorus *larvae and their moulting*

Longidorus larvae have a second stylet developing in the oesophageal wall just behind the base of the stylet extension. Larvae about to moult usually have very dense body contents and are almost black in transmitted light. The stylet of the current stage is protruded and becomes detached at its base from the stylet extension. A space appears between the current and new cuticle in the head region. The guide ring, lining of exterior amphidial ducts and stoma, are shed. While this is proceeding the new stylet passes into the lumen of the stylet extension and its posterior end becomes attached to the anterior end of the spear extension. The old cuticle is not usually ruptured until this stage is reached, when it is pierced, probably by the nematode using its new stylet, just behind the position of the old guide ring.

Comments on species of the genus Longidorus

All of the *Longidorus* spp. described above have a lip region which is continuous with, or slightly expanded from, the neck contour but not offset by a constriction. The lip region of *L. striola* Merzheevskaya, 1957 and *L. tardicauda* Merzheevskaya, 1957 is similar to that of *L. elongatus* but *L. striola* has a long tail, c = 37, and *L. tardicauda* a rounder short tail, c = 157. *L. laevicapitatus* Williams, 1959, with a continuous lip region, is characterised by its short length, L = 2-3 mm. *L. monohystera* Altherr, 1953 was described from a single female and is the only

Longidorus sp. known with a single anterior ovary. *L. nudus* Kirjanova, 1957 has a slightly offset lip region. *L. maximus* (Bütschli, 1874) Thorne & Swanger, 1936 and *L. georgiensis* Tulaganov, 1937 both have the lip region set off by a deep constriction, the main difference between them being in their length, the former is 11 mm and the latter 6 mm. *L. multipapillatus* Sch. Stek. & Teunissen, 1938 has measurements and characters which place it in *Longidorella* Thorne, 1939. However the description is inadequate and I regard it as a *species inquirenda.*

ACKNOWLEDGEMENTS

I thank Mr. P. A. A. Loof for collecting the topotypes of *L. elongatus,* Drs. N. G. Hague, B. D. Harrison, R. S. Pitcher and R. D. Winslow for supplying specimens and Misses Carole Piggott and Judith Killick for technical assistance.

ZUSAMMENFASSUNG

Eine Neubeschreibung von Longidorus elongatus *(de Man, 1876) Thorne & Swanger, 1936, (Nematoda, Dorylaimidae) und Beschreibung von 5 neuen Arten der Gattung* Longidorus *aus Gross-Britannien*

Longidorus elongatus (de Man, 1876) Thorne & Swanger, 1936 wurde nach Proben neubeschrieben, die vom Ort des Typs erhalten waren. *L. elongatus* ist charakterisiert durch den dünnen Körper, der leicht ausgedehnten vorn abgeflachten Lippenregion, dem dorsal konvexen rundlich konischen Schwanz, der ebenso lang oder ein wenig länger als die Analkörperbreite ist und durch einen 85-95 μ langen Stachel. Maße und Beschreibungen werden auch von Männchen und Larven aus Britannien gegeben.

L. leptocephalus n. sp. und *L. attenuatus* n. sp. unterscheiden sich von *L. elongatus* dadurch, dass der erstere kleiner ist mit einer engeren Lippenregion, kürzerem Stachel und mehr zugespitzten Schwanz. Der letztere hat einen noch dünneren Körper, eine stark ausgedehnte Lippenregion, einen mehr zugespitzt konischen Schwanz und einen kürzeren Stachel.

L. goodeyi n. sp. und *L. caespiticola* n. sp. sind ein wenig länger und scheinen kräftiger zu sein als *L. elongatus. L. goodeyi* hat grosse asymmetrisch zweilappige Amphiden und eine rundlich abgeflachte Lippenregion. *L. caespiticola* besitzt eine rundlich konische Lippenregion und grosse taschenartige Amphiden. Beide Arten haben breit konische bis runde Schwänze, etwa eine Analkörperbreite lang.

L. macrosoma n. sp. ist leicht erkennbar an seiner Größe, 8-10 mm, einer gleichmässig abgestutzten Lippenregion und einem kurzen runden Schwanz. Maße larvaler Stadien und Abbildungen des Schwanzes werden für jede Art gegeben.

REFERENCES

Altherr, E. (1953). Nematodes du sol Jura vaudois et français. *Bull. Soc. vaud. Sci. Nat.* **65**, (284), 429-460.

Bütschli, O. (1874). Zur Kenntnis der freilebenden Nematoden insbesondere der des Kieler Hafens. *Abh. Senkenb. naturf. Ges.* **9**, 237-292.

Goodey, T. (1951). *Soil and freshwater nematodes.* Methuen, London. 390 pp.

Kirjanova, E. S. (1951). Nematody pochvy khlipkovogo polya i tseling v Golodnoi stepi (Uzbekistan). *Trav. Inst. zool. Acad. Sci. U.R.S.S.* **9**, 625-657.

Man, J. G. de (1876). Onderzoekingen over vrij in de aarde levende Nematoden. *Tijdschr. Ned. Dierk. Ver.* **2**, 78-196.

Merzheevskaya, O. I. (1951). Novye vidy nematod. *Sborn. Nauchn. Trudov Akad. Nauk Belorussk. SSR Inst. Biol.* **2**, 112-120.

Micoletzky, H. (1922). Die freilebenden Erd-Nematoden. *Arch. Naturgesch.* (1921), **87**, 1-650.
Schuurmans Stekhoven, J. H. & Teunissen, R. J. H. (1938). Nématodes libres terrestres. *Exploration du Parc National Albert. Mission G. F. de Witte* (1933-1935). **22**, 1-229.
Thorne, G. (1939). A monograph of the nematodes of the superfamily Dorylaimoidea. *Capita zool.* **8**, 1-261.
Thorne, G. & Swanger, H. H. (1936). A monograph of the nematode genera *Dorylaimus* Dujardin, *Aporcelaimus* n.g. and *Pungentus* n.g. *Capita zool.* **6**, 1-156.
Tulaganov, A. T. (1937). Nematoden der Tomate und des sie umgebenden Bodens. *Zool. Anz.* **118**, 283-285.
Williams, J. R. (1959). Studies on the nematode soil fauna of sugar cane fields in Mauritius. 3 Dorylaimidae (Dorylaimoidea, Euoplida). *Occas. pap.* (3), *Mauritius Sug. Industr. Res. Inst.* 28 pp.

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SHORT COMMUNICATION

ETIENNE GERAERT [1]): *Corrections of measurements of nematode diameters.*

Measurements of nematode diameters have a certain systematic value and therefore should be carefully made. With few exceptions (e.g. *Wilsonema* sp.) nematodes are circular in cross section but they may be flattened in old slides where supports have not been used or in new slides where the supports are inappropriate (e.g. glass fibres used may be too thin or oval in section.) To decide whether a given nematode is flattened, the horizontal 'diameter' is measured (h) using a camera lucida or with a calibrated micrometer eye-piece and compared with the vertical 'diameter' (v), obtained by reading the calibrated micro-screw of the microscope.

The real diameter, (d), is a more or less complicated function of these two (see Fig. 1).

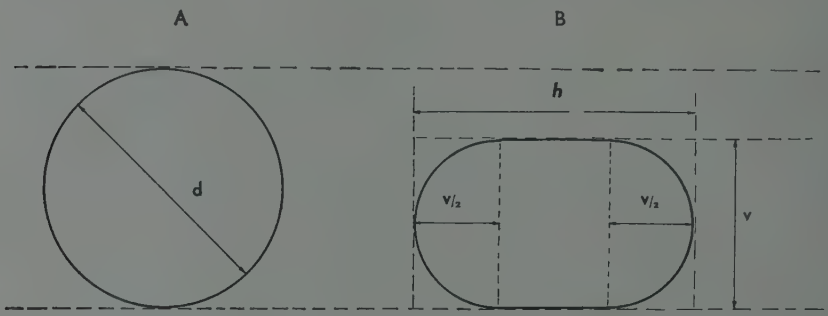

Fig. 1. scheme A: original section of a nematode; scheme B: assumed section of a flattened nematode. h = horizontal diameter; v = vertical diameter.

Three formulae may be derived: —

(1) Derived from fig. 1, no proof given

$$d_1 = \tfrac{1}{2}\,(h + v)$$

(2) Postulating that circumferences of the normal and of the flattened nematodes are the same:

$$\pi d_2 = \pi v + 2\,(h - v)$$

Approximating and taking $\pi = 3$, gives: —

$$d_2 = \tfrac{2}{3}\,h + \tfrac{1}{3}\,v$$

(3) Postulating that the circumferences differ but that the cross sectional areas remain the same: —

$$\frac{\pi}{4}\,(d_3)^2 = \frac{\pi}{4}\,v^2 + v\,(R - v)$$

Again taking $\pi = 3$, gives: —

$$(d_3)^2 = \tfrac{1}{3}\,(4\,hv - v^2)$$

Fig. 2 compares the results obtained using these formulae. It is uncertain which formula should be used because, during flattening, the volume of the nematode may or may not change. If the volume remains constant, the cuticle must be stretched and d_3 should be used or, in practice, d_1,

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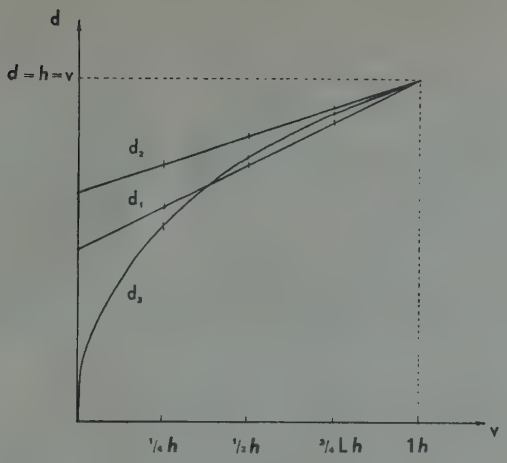

Fig. 2. Graphical representation of the variation in values of d_1, d_2, and d_3. — d (= real diameter) is plotted as ordinate against v (vertical 'diameter') as abscissa. Values of d_1, d_2 and d_3 have been calculated for $v = 1/4\ h$, $1/2\ h$, $3/4\ h$ and $1\ h$ according to the equations, h being the horizontal 'diameter'. At $v = 1\ h$ there is no compression so that d, v, and h are all equal to each other.

which varies very much as d_3 (see Fig. 2). If the volume varies, the cuticle is less stretched and d_2 should be used.

Although no work has been done on change of volume with flattening, it seems reasonable to suppose that volume diminishes on flattening. The contents of the gut are subject to pressure and may exude at mouth and anus. In dead animals the cuticle may be more permeable so that substances of low molecular weight may pass out through it also tending to reduce the volume.

I thank Dr. J. W. Seinhorst and Professor de Coninck for their advices.

NOTICE

International Commission on Zoological Nomenclature. Notice of proposed use of plenary powers in certain cases.

In *Bull. zool. Nomencl.* **18** (3), 206-7 Loof puts forward a case asking the International Commission on Zoological Nomenclature to suppress the specific name *gulosus* Kühn, 1890 as published in the binomen *Tylenchus gulosus* for the purposes of the Law of Priority and to place the specific name *penetrans* Cobb, 1917, as published in the binomen *Tylenchus penetrans* on the Official List of Specific Names in Zoology. Comments (in duplicate) are asked from interested nematologists to be sent to the Secretariat as soon as possible, and in any case to reach the Secretariat within six months of 16th June, 1961, the date of publication of Loof's case.

All comments should be addressed to: —

The Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London, S.W. 7, England.

BOOK REVIEW

Dr. A. C. Tarjan is to be thanked for compiling this *Check List of Plant and Soil Nematodes* and congratulated on the excellent quality of the work. It is authoritative and accurate in presenting taxonomic data but free from bias and leaves the reader to follow his own particular line in systematics.

As the title implies, it deals with plant and soil nematodes and the names of about 3,000 species, subspecies, varieties and forms are considered. Under each specific name is a chronological list of entries starting with the original genus in which the species was placed and followed by generic names and synonyms to which the species has been transferred. Each entry has a reference to the transferring authority and occasionally there is a brief comment by Dr. Tarjan indicating the opinion of some specialist. Marine, animal-parasitic and insect-parasitic nematodes have not been considered which is reasonable enough but, particularly in the Chromadorids, this limitation has drawbacks because those few species occurring in soil or freshwater are seen out of context with their nearest relatives. As a general comment, not directed to Dr. Tarjan, it is unfortunate that the study of the Nematoda is now split into four separate disciplines: — (1) free-living and plant parasitic; (2) animal helminths; (3) insect parasites and (4) marine nematodes. The taxonomy and systematics of the whole of the Nematoda is not helped forward by this present schizoid state.

The presentation is pleasing and remarkably free from errors and omissions. Detailed use has brought to light under twenty such items but these are easily checkable.

A very valuable part of the work lies in the cited literature where about 900 references are given. It is greatly to be hoped that Dr. Tarjan will continue this work and issue supplements from time to time and perhaps, in due course, cover the field of insect parasites and the more limited field of those nematodes living in freshwater or brackish habitats whose relatives are marine. The Check List is published by the University of Florida Press, Gainesville, Florida, U.S.P. at $ 7.50, a reasonable price for the work.

14th April, 1961

J. B. Goodey
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NEMATODE TRACKS IN THE STUDY OF MOVEMENT OF *MELOIDOGYNE INCOGNITA INCOGNITA* [1])

BY

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Tracks of the plant-parasitic nematode, *Meloidogyne incognita incognita*, were obtained on a nutrient 2% agar medium from which free surface water had been allowed to evaporate. Under the experimental conditions used there was no apparent polarity of the tracks toward actively growing tomato roots or seedlings. The larvae apparently made contact with the roots by random and constant movement.

Larvae moving forward were unaffected by their own or other nematode tracks; in moving backward for short distances they followed the original tracks made when moving forward. The track of an adult male was also obtained.

Attraction of plant-parasitic nematodes toward plant roots has been claimed by many workers, most notably Bird (1959); Linford (1939); Lownsbery & Viglierchio (1960); Peacock (1959); Viglierchio (1961); Wallace (1960); and Widdowson, Doncaster & Fenwick (1958). Other workers (Bergman & van Duuren, 1959; Kühn, 1959; and Staar, 1959), however, have concluded that nematodes are not attracted to host roots from a distance. Usually the alternative hypothesis has been that nematodes reach the root by random movement. Wallace (1958a, 1958b, 1959a, 1959b) has drawn the paths of movement of nematodes with the aid of a camera lucida and has taken cinemicrographs of nematodes moving in water films. Staar (1959) has observed and photographed visible, aseptic tracks of *Heterodera rostochiensis* Woll. on 2% agar.

This paper explains our method of obtaining tracks of *Meloidogyne incognita incognita* (Kofoid and White); photographs of the tracks illustrate the random nature of movement, even near growing roots.

MATERIALS AND METHODS

White's nutrient agar medium as modified by Skoog & Tsui (1948) was used. It had the following composition (concentrations in mg liter): $MgSO_4.7H_2O$ 72.0, $Ca(NO_3)_2.4H_2O$ 144.0, KNO_3 80.0, NH_4NO_3 400.0, KH_2PO_4 38.0, KCl 65.0, $ZnSO_4.7H_2O$ 2.7, $MnSO_4.H_2O$ 4.9, H_3BO_3 1.6, KI 0.75, nicotinic acid 0.5, pyridoxine hydrochloride 0.5, thiamin hydrochloride 0.1, glycine 2.0, sucrose

[1]) Published with the approval of the Director as Paper No. 1106, Journal Series, Nebraska Agricultural Experiment Station.

20,000, and agar (Albimi Laboratories) 20,000. Iron (1×10^{-4}M) was added as ferric potassium ethylenediamine-tetraacetate. Pyrex distilled water was used throughout. The pH of the medium was adjusted to 6.2-6.5 with NaOH. The medium was then autoclaved for 20 minutes at 2 atmospheres and poured into sterile plastic petri dishes, size 60 × 15 or 100 × 15 mm. The prepared dishes were stored for 1 week at 23-25° C to allow all traces of free water to evaporate from the surface.

Seeds of tomato (*Lycopersicon esculentum* Mill.), 'Kokomo' (Asgrow), were sterilized by aspiration in 10% Purex (a commercial bleach containing 5.25% NaOCl) for 20 minutes. They were then rinsed with sterile pyrex-distilled water and placed on moistened filter paper in petri dishes to germinate. When the radicles were ca. 15 mm long, the terminal 10 mm was excised and placed on the surface of the agar medium near one side of the dish. In some experiments the intact seedling was placed on the medium when the radicle was 1 or 2 mm long. The root and seedling cultures were grown in an incubator at 28° C in the dark.

The excised roots grew at a rate of ca. 14 mm/day and the seedling radicles grew ca. 17 mm/day. The hardness of the agar (2%) prevented the roots from penetrating beneath the surface. After the roots or seedlings had been on the medium for 1-4 days (the longer duration could be used with the larger sized dishes before the root reached the edge of the dish) an excised aseptic gall or egg sac of *Meloidogyne incognita incognita* was placed on the medium in the center of the dish. The galls and egg sacs were obtained from aseptic cultures of the nematode growing in tomato roots as described by Loewenberg, Sullivan & Schuster (1960).

The larvae usually began to hatch immediately after transfer and to move over the surface of the medium. Tracks made by the nematodes could be seen by transmitted light with an ordinary low power (30 ×) microscope or by light reflected from the surface of the agar without the aid of a microscope. Photographs of the tracks were taken on Kodak Panatomic-X film and developed with Kodak D-19 developer for maximum contrast.

RESULTS

Typical nematode tracks are shown in Figures 1-3. Figure 1 shows the track of a single nematode (A). The track had a wavy shape which conformed with the shape of the nematode's body during motion. For the track to be in focus, the nematode had to be slightly out of focus which made it look wider than it actually was. At point B the nematode reversed itself for a short distance. When moving backward it followed the track originally made when moving forward. This was a common occurrence and can also be seen in the other figures. Nematodes moving forward, however, did not follow in their own or in other nematode's tracks.

Figure 2 shows nematode tracks near the root tip (A) of an intact seedling.

R. SANDSTEDT, Thelma SULLIVAN & M. L. SCHUSTER: *Nematode tracks in the study of* Meloidogyne.

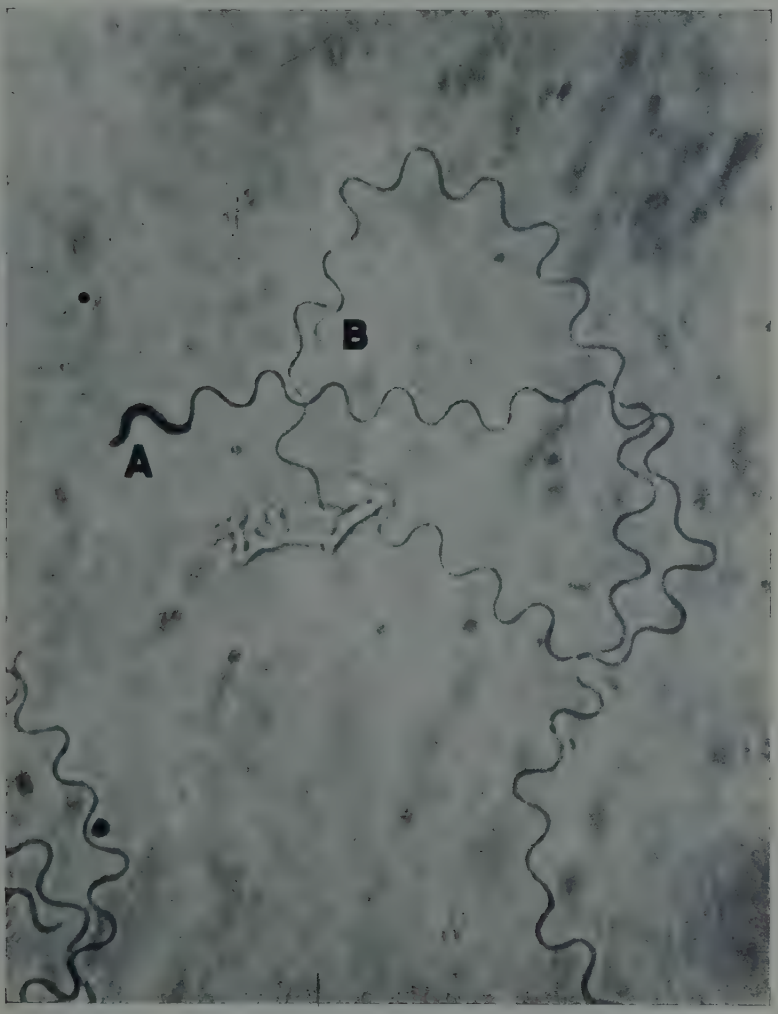

Figure 1. Typical track of a *M. incognita incognita* larva on nutrient agar surface. Length of larva $^{1}/_{3}$ mm.

Figure 2. Nematode tracks on nutrient agar surface near the root tip of a tomato seedling. Diameter of field, 5 mm.

Figure 3. Larval and adult male tracks on nutrient agar surface in petri dish with excised tomato root tip. Diameter of field, 5 mm.

The root tip appears slightly bulbous because the microscope was focussed on the tracks. The seedling had been on the medium for 27 hours and the egg sac for 3 hours when the picture was taken. Although a nematode approached to within 1 mm of the root, it did not make contact at that time. The nematode reversed its direction at B and C and several other places. The tracks appeared to be the result of aimless wandering.

Figure 3 shows tracks of larvae, some of which had made contact with an excised root (A). The root had been on the medium 31 hours and the gall 4 hours when the picture was taken. The heavy track (B) across the middle of the field was made by an adult male which came from the same gall as the larvae. Adult males were rarely observed. A great deal of side slippage is evident in the adult male's track. Occasionally in these studies, a larva was observed to leave the root; one did so at C. Two other larvae can be seen near the right edge of the field. Again, the tracks showed no orientation of direction.

After a gall or egg sac had been on the center of the medium for a day or more, the entire surface of the medium was usually covered with a network of nematode tracks. The tracks were equally numerous on the side of the petri dish toward and away from the root. This could be observed both through the microscope and with the naked eye. Most larvae which contacted the root entered and stimulated the formation of galls.

DISCUSSION

Nematode tracks provide a record of nematode movement under aseptic conditions on a homogeneous, chemically-defined medium. Agar is well suited for studies involving diffusion of root exudates. The method is direct; the larvae and their tracks can be seen at any time. Possible confusion between positive attraction and mere detention of larvae is avoided.

Tracks made by larvae of *M. incognita incognita* indicated that the nematodes were not attracted toward tomato roots on agar from any distance, but reached the roots by moving randomly but continuously until they happened to make contact. This evidence is compatible with the study of *Heterodera rostochiensis* tracks by Staar (1959). Kühn (1959), using a different method, reached similar conclusions. Both workers have postulated that the building up of diffusion gradients of an attractant substance in soil is almost impossible.

The possibility exists that the 2% agar prevented ready diffusion of postulated attractant materials from the root in these experiments. However, the medium was soft enough that nematode larvae could make tracks on it while moving about freely; excised tomato roots, for which the medium was the only source of nutrients and water, grew vigorously on the surface; larvae emerged from egg sacs and galls immediately on being transferred, presumably due to stimulation from the medium; and when crystals of picric acid were sprinkled on the surface of such medium, they dissolved and diffused, as judged by the diameter of the color spot, at 4 mm hour. Since these phenomena took place on the surface of the agar, it is

likely that diffusion of hypothetical attractants from the roots also could take place on the same surface.

Why have nematode tracks on agar been reported only recently? When eggs or larvae in water are pipetted onto an agar medium, as is often done, the water lubricates the surface so that no tracks are made. Even when the nematodes move out from the area of surface water they still are often surrounded by a tiny pool of water which clings to them by surface tension. Under such conditions the larvae will not leave tracks until the water has evaporated. Some workers have used semi-solid agar medium so that the tracks were probably covered over. Staar (1959) observed tracks of *Heterodera rostochiensis* through the microscope only with special lighting and apparently not with the unaided eye. He did not report if he added the larvae in a water slurry or otherwise.

It should be mentioned that tracks on agar made by aseptic nematodes are quite different from the microbe trails left by non-sterile nematodes as reported by Kalinenko (1936).

Further studies of nematode movement with tracks are in progress including effects of nutrients, pH, light, nematode virus (Loewenberg, Sullivan & Schuster, 1959) and different species of roots and seedlings with *M. incognita incognita* as well as other nematode species. As the tracks can be seen with the unaided eye, it should be possible, with the solving of technical difficulties, to photograph their pattern over the entire surface of a 60 mm petri dish.

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ZUSAMMENFASSUNG

Nematodenspuren bei Untersuchungen über die Bewegung von Meloidogyne incognita incognita. Spuren von *Meloidogyne incognita incognita* liessen sich auf 2% Nähragar feststellen, von dem das Oberflächenwasser verdampft worden war. Unter den experimentellen Bedingungen zeigte sich keine Polarität in Richtung kräftig wachsender Tomatenwurzeln oder -sämlinge. Die Larven kamen augenscheinlich mit den Wurzeln durch ziellose und fortwährende Bewegung in Berührung.

Sich vorwärtsbewegende Larven waren unbeeinflusst durch eigene Spuren oder Spuren anderer Nematoden. Soweit sie sich aber über kurze Strecken rückwärts bewegten, folgten sie den Spuren, die bei ihrer eigenen Vorwärtsbewegung entstanden waren. Die Spur eines erwachsenen Männchens wurde auch beobachtet.

LITERATURE CITED

Bergman, B. H. H. & van Duuren, A. J. (1959). Het bietencystenaaltje en zijn bestrijding. VI. De invloed van wortels van waardplanten en excreten hiervan op de bewegingsrichting van larven van *Heterodera schachtii* in vitro. *Meded. Inst. Suikerbiet., Bergen-o.-Z.* **29**, 1-24.

Bird, A. F. (1959). The attractiveness of roots to the plant parasitic nematodes *Meloidogyne javanica* and *M. hapla*. *Nematologica* **4**, 322-335.

Kalinenko, V. O. (1936). The inoculation of phytopathogenic microbes into rubber-bearing plants by nematodes. *Phytopath. Z.* **9**, 407-416.

Kühn, H. (1959). Zum Problem der Wirtsfindung phytopathogener Nematoden. *Nematologica* **4**, 165-171.

Linford, M. B. (1939). Attractiveness of roots and excised shoot tissues to certain nematodes. *Proc. helm. Soc. Wash.* **6**, 11-18.

LOEWENBERG, J. R., SULLIVAN, T., & SCHUSTER, M. L. (1959). A virus disease of *Meloidogyne incognita incognita,* the southern root-knot nematode. *Nature Lond.* **184**, 1896-1897.

——, —— & —— (1960). Gall induction by *Meloidogyne incognita incognita* by surface feeding and factors affecting the behavior pattern of the second-stage larvae. *Phytopathology* **50**, 322-323.

LOWNSBERY, B. F. & VIGLIERCHIO, D. R. (1960). Mechanism of accumulation of *Meloidogyne incognita acrita* around tomato seedlings. *Phytopathology* **50**, 178-179.

PEACOCK, F. C. (1959). The development of a technique for studying the host/parasite relationship of the root knot nematode *Meloidogyne incognita* under controlled conditions. *Nematologica* **4**, 43-55.

SKOOG, F. & TSUI, C. (1948). Chemical control of growth and bud formation in tobacco stem segments and callus cultured *in vitro*. *Amer. J. Bot.* **35**, 782-787.

STAAR, G. (1959). Über einige Ergebnisse aus reizphysiologischen Versuchen an *Heterodera rostochiensis*. *Deutsche Akad. Landwirtschaftswiss. Berlin, Tagungsber.* **20**, 49-52.

VIGLIERCHIO, D. R. (1961). Attraction of parasitic nematodes by plant root emanations. *Phytopathology* **51**, 136-142.

WALLACE, H. R. (1958a). Movement of eelworms. I. The influence of pore size and moisture content of the soil on the migration of larvae of the beet eelworm, *Heterodera schachtii* Schmidt. *Ann. appl. Biol.* **46**, 74-85.

—— (1958b). Movement of eelworms. II. A comparative study of the movement in soil of *Heterodera schachtii* Schmidt and of *Ditylenchus dipsaci* (Kühn) Filipjev. *Ann. appl. Biol.* **46**, 86-94.

—— (1959a). Movement of eelworms. V. Observations on *Aphelenchoides ritzema-bosi* (Schwartz, 1912) Steiner, 1932 on florists' chrysanthemums. *Ann. appl. Biol.* **47**, 350-360.

—— (1959b). The movement of eelworms in water films. *Ann. appl. Biol.* **47**, 366-370.

—— (1960). Movement of eelworms. VI. The influences of soil type, moisture gradients and host plant roots on the migration of the potato-root eelworm *Heterodera rostochiensis* Wollenweber. *Ann. appl. Biol.* **48**, 107-120.

WIDDOWSON, E., DONCASTER, C. C., & FENWICK, D. W. (1958). Observations on the development of *Heterodera rostochiensis* in sterile root cultures. *Nematologica* **3**, 308-314.

WIESER, W. (1955). The attractiveness of plants to larvae of root-knot nematodes. I. The effect of tomato seedlings and excised roots on *Meloidogyne hapla* Chitwood. *Proc. helm. Soc. Wash.* **22**, 106-112.

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DIE PHOTOGRAPHISCHE DARSTELLUNG DER KRIECHSPUREN (ICHNOGRAMME) VON NEMATODEN UND IHRE BEDEUTUNG

VON

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Nach einer kurzen Diskussion einiger Probleme, die sich aus reizphysiologischen Untersuchungen an Larven von *Heterodera rostochiensis* Woll. ergaben, wird eine Methode beschrieben, mit deren Hilfe das Verhalten der Tiere gegenüber chemischen und physikalischen Reizen durch Sichtbarmachung der Kriechspuren auf Agar unter bestimmter Auflichtführung visuell und photographisch bzw. kinematographisch in allen Einzelheiten verfolgt werden kann.

Um über das Zustandekommen der Wurzelfindung bei freilebenden Larven des Kartoffelnematoden *Heterodera rostochiensis* Wollenweber etwas Endgültiges aussagen zu können, genügt es offenbar nicht, die örtliche Verteilung der Versuchspopulation am Ende einer bestimmten Expositionszeit numerisch auszuwerten. Die Ansammlung von Larven an den Wurzeln läßt sich auf verschiedene Weise erklären. Sie könnte z.B. die Folge einer Art Fallenwirkung sein dergestalt, daß die Tiere den unmittelbaren Bereich der Wurzeln nicht mehr verlassen, wenn sie zufällig einmal hineingeraten sind. Denkbar ist auch, daß ein von den Wurzeln ausgehender Reiz lediglich die lokomotorische Agilität der Larven stimuliert und daß damit die Wahrscheinlichkeit des im übrigen rein zufälligen Zusammentreffens mit einer Wurzel erhöht wird. In ähnlicher Weise deutete schon Lane (1930) bei Thermoreizversuchen von Fülleborn (1924) das Verhalten von Hakenwurmlarven.

Eine weitere Erklärungsmöglichkeit bietet die Annahme einer chemotaktischen Orientierung, die sowohl auf phobischer wie auf topischer Reaktion beruhen könnte. Hierzu müßte allerdings das Vorhandensein eines von den Tieren wahrnehmbaren chemischen Reizgefälles vorausgesetzt werden. Ein solches ist jedoch unter natürlichen Verhältnissen im Boden außer in unmittelbarer Umgebung einer Wurzel aus verschiedenen Gründen (z.B. Bodenstruktur und Wasserbewegung, Überschneidung hypothetischer Reizgefälle benachbarter Wurzeln, mikrobieller Abbau der Reizsubstanzen u.a.m.) nicht recht vorstellbar, worauf schon von Staar (1959) und Kühn (1959) hingewiesen wurde.

Nichtsdestoweniger könnten die Tiere unter bestimmten Voraussetzungen vielleicht doch in der Lage sein, sich chemotaktisch zu orientieren. Wir beobachteten

in Modellversuchen mit Agarschichten daraufhin das Verhalten von Einzeltieren anhand von Standortsbestimmungen in regelmäßigen Zeitabständen. Als Reizquelle fungierte jedesmal das Wurzelsystem eines auf dem Agar herangezogenen Kartoffelkeimes, das sich innerhalb eines "Käfigs" aus feinem Perlongewebe (Maschenweite 65 μ) an dessen Periphere als mehr oder weniger dichter Filz ausbreitete. Die Gazeschranke behinderte nicht die Ausbreitung der Wurzelausscheidungen, führte aber zu einer recht scharfen, unveränderlichen Begrenzung

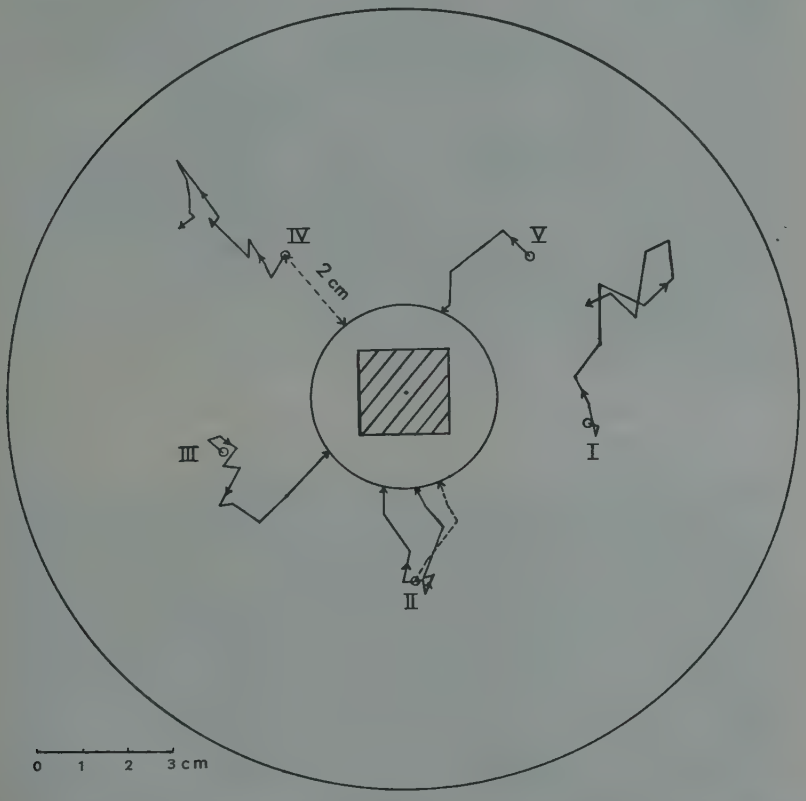

Fig. 1. Rekonstruktion der Wanderbahnen anhand von intermittierenden Standortsbestimmungen (20'). An Punkt II wurden drei Larven nacheinander eingesetzt. Die bei I und IV eingesetzten Larven haben den zuletzt ermittelten Standort nicht mehr verlassen.

der Reizquelle. Die Versuche begannen jeweils nach Ausbildung eines genügend entwickelten Wurzelsystems, d.h. ca. 3 bis 5 Wochen nach dem Ansetzen des Kartoffelkeims.

Aus den Standortsbestimmungen (Fig. 1) ergab sich, daß sich die einzelnen Tiere nicht zielstrebig auf kürzestem Wege zur Reizquelle hinbewegten. Zwar

bevorzugte die Mehrzahl von ihnen bei Übertragung auf die Agarfläche in einem Abstand von weniger als 3 cm von der Reizquelle deutlich die positive, d.h. zum Wurzelfilz führende Wanderrichtung, doch bewegten sie sich in wechselnder Richtung und auf Bahnen, die sie zumindest zeitweise wieder von der Reizquelle entfernten. Es zeigte sich, daß die Wanderbahnen mit mehr oder minder großem Radius rückläufig werden und sich dabei u.U. selbst wieder überschneiden können. Wiederholt werden eigenartige „Wirrspuren" eingeschaltet. An diesen Stellen wird die Vorwärtsbewegung durch eine irreguläre Bewegung „am Ort" abgelöst. Nach einiger Zeit verläßt die Larve die „Wirrspur" und wandert in irgendeiner Richtung weiter (s.u.). Merkwürdigerweise änderte sich an diesem Verhalten auch bei Annäherung an die Gazeschranke, also die effektive Reizquelle, in der Regel nichts.

Zur näheren Untersuchung des Verhaltens von Larven des Kartoffelnematoden oder auch anderer Nematoden im Bereich einer chemischen oder einer anderen Reizquelle müßte der Wanderweg einzelner Älchen k o n t i n u i e r l i c h verfolgt werden können. Derartige fortlaufende Kontrollen sind aber mit dem Nachteil verbunden, daß sich dabei die Einwirkung anderer Faktoren, z.B. thermischer Gefälle, störend bemerkbar machen kann. Das läßt sich vermeiden, wenn man die Versuche von vornherein so anlegt, daß sie störungsfrei ablaufen und Ichnogramme liefern, die sichtbar bleiben und nach Beendigung der Expositionszeit ausgewertet werden können.

Das Berußen der Agarfläche und das Aufbringen einer feinen Staubschicht (Glas- oder Karborundpulver u.ä.) führte nicht zum Ziel. Dagegen entstehen unter bestimmten Voraussetzungen bei der Fortbewegung der Tiere auf der Agarfläche bleibende Kriechspuren in Form rillenartiger Bahnen. Diese Ichnogramme sind jedoch auch bei starker Vergrößerung nicht ohne weiteres zu erkennen, werden aber sichtbar, sobald man die Agarplatte unter einem Präpariermikroskop mehr oder weniger stark neigt und bei steil einfallendem Auflicht betrachtet. Zur allgemeinen Orientierung über das Vorkommen einzelner Spuren ist dieses primitive Verfahren noch geeignet, für die eingehendere Untersuchung wirkt sich jedoch die neigungsbedingte Unschärfe im Bildfeld und die Schwierigkeit einer ununterbrochenen Verfolgung der wandernden Tiere sehr ungünstig aus. Mit Hilfe der nun zu beschreibenden Methode ist es, abgesehen von der bequemen visuellen Beobachtung, möglich, die Ichnogramme zu photographieren und ihre Entstehung kinematographisch darzustellen. Letzten Endes ist es möglich, den Wanderweg eines Tieres in seinem ganzen Verlauf gleichsam kartographisch aufzunehmen und ihn hinsichtlich aller Richtungsänderungen zu vermessen. Unter bestimmten Voraussetzungen und mit einem gewissen erhöhten apparativen Aufwand kann auch der zeitliche Verlauf der Wanderung intermittierend bzw. kontinuierlich dargestellt werden.

Das Wesentliche an dieser Methode ist die kritische Auflicht-Führung. Ein horizontales paralleles Lichtbündel wird mit Hilfe eines kleinen Spiegels annähernd senkrecht auf die zu betrachtende Fläche gelenkt. Die infolge Lichtreflexion an

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Fig. 2. Auflicht-Apparatur für die Untersuchung der Kriechspuren von Nematoden.

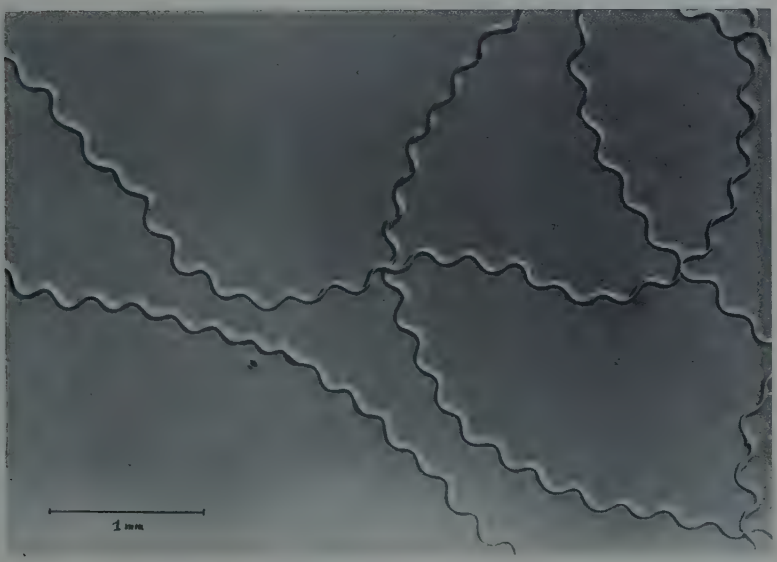

Fig. 3. Ichnogramm von *Heterodera rostochiensis.*

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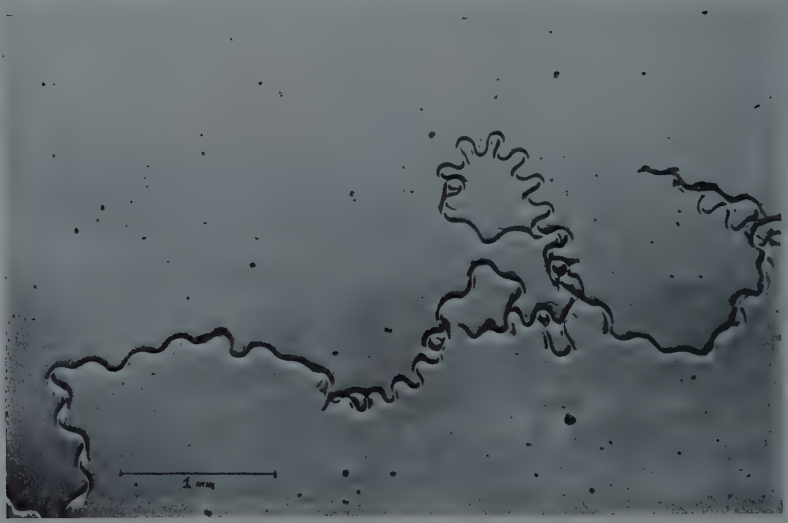

Fig. 4. Ichnogramm von *Heterodera rostochiensis* im thermischen Gefälle.

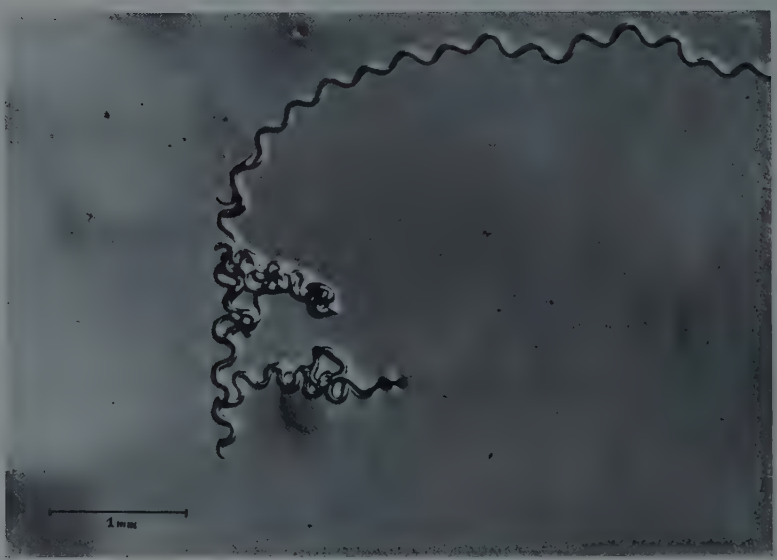

Fig. 5. „Wirrspuren" von *Heterodera rostochiensis.*

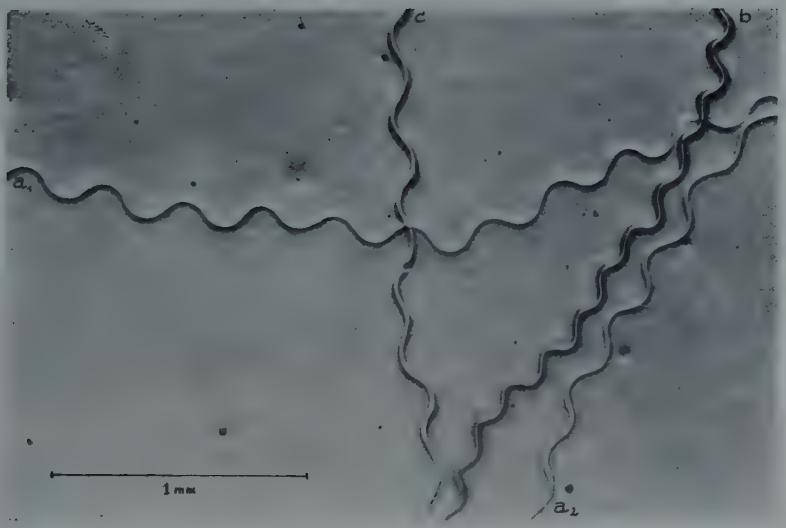

Fig. 6. Ichnogramme von *Heterodera rostochiensis* (a_1, a_2) und *Aphelenchoides ritzemabosi* (b junges Tier, c älteres Tier).

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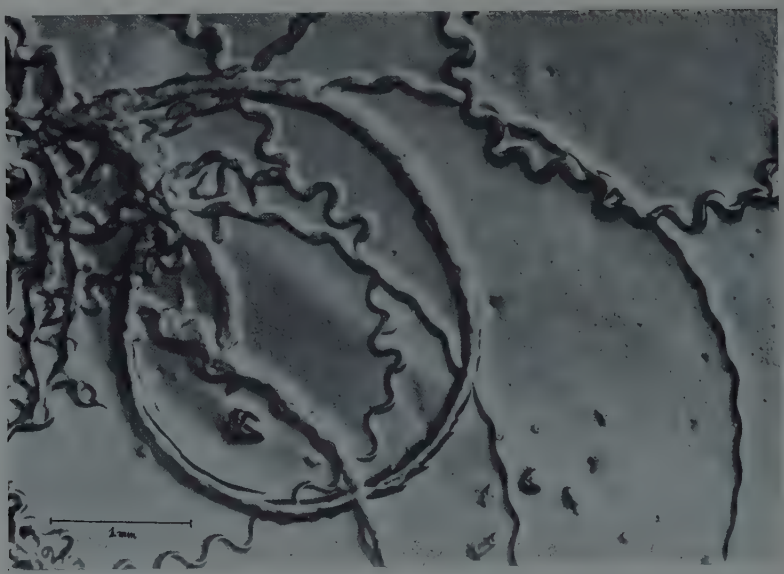

Fig. 7a. Ichnogramme eines indeterminierten Nematodengemisches aus dem Bereich einer faulenden Wurzel von *Humulus lupulus.*

Fig. 7b. Ichnogramme eines indeterminierten Nematodengemisches aus dem Bereich einer faulenden Wurzel von *Humulus lupulus.*

ihren konkaven Wandungen aufleuchtenden Kriechspuren werden dicht am Auflichtspiegel vorbei durch ein etwas gegen die Senkrechte geneigtes optisches System mit großem Arbeitsabstand betrachtet.

Bei Verwendung des Stereomikroskops „Citoplast" (Zeiss-Jena) lassen sich die geforderten Voraussetzungen relativ leicht schaffen (Fig. 2). Ein Grundbrett von 60 cm Länge und 22 cm Breite wird an beiden Enden mit 2,5 cm hohen Fußleisten versehen, sodaß die Grundplatte des „Citoplast" daruntergeschoben werden kann. Am linken Ende des Grundbrettes wird eine verstellbare Brücke mit einer lichten Höhe von ca 2,5 cm aufgesetzt. Auf dieser Brücke längsverschiebbar ist ein Schlitten angebracht, der die Halterung für den Spiegel trägt. Mit deren Hilfe soll der Spiegel in einem Bereich von 4 cm vertikal verstellbar sein.

Der Silberspiegel selbst wird nach einem der bekannten Rezepte aus einem Mikroskopier-Deckgläschen (10 × 10 mm) hergestellt und am Ende eines dünnen, 20 cm langen Glasstabes so angekittet, daß er um eine Kante geschwenkt werden kann. Der Glasstab wird in einem 14 cm langen, möglichst genau passenden Glasrohr unter Zugabe von etwas Vaseline oder Gleitfett gelagert, sodaß er mit mäßiger Hemmung drehbar und zugleich längsverschiebbar ist. Das freie Ende wird zu diesem Zweck auf ungefähr 3 cm rechtwinklig abgebogen oder mit einer Rundscheibe von 6 cm Durchmesser versehen. Das Ganze läßt sich dann mit Hilfe eines halbierten Kork- oder Gummistopfens ohne Schwierigkeiten in der erwähnten Haltevorrichtung befestigen. Der Spiegel kann nun nach drei Richtungen hin verstellt und hinreichend genau justiert werden.

Als Lichtquelle verwenden wir eine starke Mikroskopierleuchte (ohne Mattglas), die dem Beobachter gegenüber, also hinter dem Präpariermikroskop aufgestellt wird. Ihr parallel gehaltenes Lichtbündel fällt genau horizontal und außerdem senkrecht zur Drehachse auf den Spiegel.

Genau so kritisch wie die Lichtführung ist die Beschaffenheit der Agaroberfläche. Sie muß abgetrocknet und ein wenig verdichtet sein. Wir verfahren folgendermaßen. Der Agar wird mit aqua dest. 2 %ig angesetzt, über Watte heiß filtriert, bei 1,5 atü im Autoklaven 20 Min. erhitzt und nach Abkühlung auf 50° C in einer nur wenige Millimeter dicken Schicht in flache Petrischalen, Metallrahmen o.ä. ausgegossen. Bis zur Erstarrung bleiben die Schalen bzw. Rahmen zugedeckt, dann zur Abtrocknung und leichten Verdichtung der obersten Agarschicht gegen Staub geschützt bei 20° C offen stehen. Die dazu notwendige Zeit ist je nach Agarmaterial und Schichtdicke verschieden und muß ausprobiert werden. Nach unseren Erfahrungen handelt es sich um eine halbe bis höchstens zwei Stunden. Anzustreben ist die Ausbildung einer möglichst ebenen Agarfläche. Die Eignung verschiedenen Agarmaterials ist offenbar recht unterschiedlich, denn manches davon neigt beim Abtrocknen in dünner Schicht sehr zur Ausbildung einer stark gewellten, mitunter sogar geriffelten Oberfläche.

Zur visuellen Beobachtung wie zur photographischen bzw. kinematographischen Abbildung der Kriechspuren ist es zweckmäßig, der Agarschicht einen gleichmäßig dunklen oder schwarzen Hintergrund zu geben.

Die Leistungsfähigkeit der hier beschriebenen Methode wird durch Fig. 3 bis 7 demonstriert. Fig. 3 zeigt charakteristische Ichnogramme einiger Larven von *Heterodera rostochiensis* bei gleichmäßig ruhiger Bewegung in einem reizhomogenen Feld. Es sei kurz darauf hingewiesen, daß die abgebildeten Spuren infolge optischer Täuschung vertieft, wie es der Wirklichkeit entspricht, oder fadenförmig aufliegend erscheinen können. Fig. 4 gibt einen 5 mm langen Abschnitt (Luftlinie) aus einem insgesamt 8 cm langen Wanderweg eines einzelnen Kartoffelälchens im thermischen Reizfeld wieder (Das Temperaturgefälle betrug innerhalb des abgebildeten Bereiches 0,1° C). Die Larve hat sich keineswegs immer zielgerichtet vorwärts bewegt. Ihre Wanderrichtung zeigt sogar wiederholt eine rückläufige Tendenz. Besonders interessant ist die mittlere Partie dieses Ichnogramms. Hier hat die Larve mehrmals nach einer scharfen Kehrtwendung ihre Vorwärtsbewegung abgebrochen und ist dann eine gewisse Strecke mit der Schwanzspitze voran, also rückwärts weitergewandert. In solchen Fällen ist stets eine auffallende Verkürzung der „Wellenlänge" des Ichnogramms zu beobachten. Hinzuweisen ist ferner auf die im ganzen Verlauf der Wanderspur auftretenden, von uns sogenannten „Überschläge" (overshoots). Es erweckt zunächst den Anschein, als sei an diesen Stellen das Schwanzende des Tieres an der Wand der vertieften Kriechspur abgeglitten und über deren Rand hinausgeschlagen. Mit der nach der üblichen Auffassung streng in einer Ebene verlaufenden Schlängelung des Älchenkörpers sind diese „Überschläge" aber nicht recht in Einklang zu bringen. Fig. 5 zeigt zwei der zuvor beschriebenen „Wirrspuren". Das Älchen ist von rechts oben her in das Bildfeld eingewandert und bewegte sich während der photographischen Exposition am äußeren Ende der zweiten „Wirrspur" rückwärts.

Daß die Ichnogramme von Nematoden u. U. auch eine gewisse diagnostische Bedeutung bekommen können, geht aus Fig. 6 und 7 a/b hervor. Auffallende Unterschiede sind sowohl in der Grundform als auch im Gesamtverlauf der Kriechspuren erkennbar. Die Charaktere scheinen artspezifisch zu sein.

Unsere Arbeiten über das reizphysiologische Verhalten von Nematoden werden anhand der hier beschriebenen Methode und unter Zuhilfenahme der Kinematographie fortgesetzt.

SUMMARY

The Photographic Demonstration of Nematode Tracks and their Significance

In studying the reaction of nematodes to attractant substances, recording the distribution of the population in relation to the source at the end of a given time is unsatisfactory. The movements of individuals need to be traced continuously. Under certain conditions furrow-like tracks are left on agar disks and by, suitable incident illumination, can be seen and photographed or filmed. By means of a small mirror a horizontal beam of parallel light rays is directed almost vertically on the agar plate to be examined. The sinusoidal tracks become visible because of light reflected from their concave sides and can be observed through a stereomicroscope with long working-distance objective, if the optical axis is slightly inclined from the vertical. The tracks are more easily seen against a dark background.

The medium used was 2 % agar poured out in thin layers. After settling, the agar disks were kept uncovered at room temperature for a time, to dry the surface slightly, but were protected from dust.

LITERATUR

FÜLLEBORN, F. (1924). Über 'Taxis' (Tropismus) bei Strongyloides- und Ankylostomen-Larven. *Arch. Schiffs u. Tropenhyg.* **28**, 144.

KÜHN, H. (1959). Zum Problem der Wirtsfindung phytopathogener Nematoden. *Nematologica* **4**, 165-171.

LANE, C. (1930). Behavior of infective Hookworm Larvae. *Ann. trop. Med. Parasit.* **24**, 411-421.

STAAR, G. (1959). Über einige Ergebnisse aus reizphysiologischen Versuchen an *Heterodera rostochiensis*. *Tagungsber. Dtsch. Akad. Landw. Wiss. Berlin,* **20**, 49-52.

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HETERODERA ORYZAE N. SP. (NEMATODA-TYLENCHOIDEA) PARASITE DU RIZ EN CÔTE D'IVOIRE

PAR

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Les auteurs décrivent *Heterodera oryzae* n. sp., rencontré sur riz pluvial de la variété Morobérékan à Bokakouamékro près de Bouaké (Côte d'Ivoire). Cette espèce, du groupe *schachtii*, se caractérise par des kystes brun foncé à noirs, limoniformes, mesurant 0,31-0,81 × 0,22-0,69 mm (0,57 × 0,45 mm en moyenne), avec cône vulvaire net et ornementation superficielle en zigzags non orientés. Les juvéniles au stade 2 mesurent 0,375 à 0,507 mm (moyenne 0,440 mm) avec un stylet de 19,5-22 μ; ils sont caractérisés par leur champ latéral à trois lignes seulement et une extrémité effilée avec portion hyaline beaucoup plus longue que le stylet (35-45 μ). Peu de mâles ont été rencontrés; ils ressemblent à ceux de *H. schachtii*.

Au cours d'une tournée dans la région nord de la Côte d'Ivoire l'un des deux auteurs avait rapporté des racines de riz (*Oryza sativa* L.) arrivé à maturité et apparemment sain. Un examen „pour voir" des racines fut cependant fait qui révéla la présence d'un *Heterodera* nouveau décrit ici sous le nom de *Heterodera oryzae* n. sp.

Ce riz provenait du village pilote de Bokakouamékro, à 40 km à l'Est de Bouaké. Le terrain sur lequel est établie la rizière est un sol de bas fond, gris, argileux, très humifère. Le riz cultivé est du riz pluvial à cycle de 120 jours. L'emplacement de la rizière actuelle a déjà été cultivé en riz vers 1945; après quelques années cette culture a été abandonnée. Le terrain fut de nouveau défriché en 1959; une première culture de riz, de la variété Gambiaka, a eu lieu d'août 1959 à janvier 1960. Pour la deuxième culture, d'août 1960 à janvier 1961, la variété Moroberekan fut utilisée.

La présence d'espèces du genre *Heterodera* dans les zones subéquatoriales d'Afrique n'a été signalée que très récemment (Luc, 1961).

HETERODERA ORYZAE N. SP.

Mâles (Fig. 1, D, E, G)

Dimensions (5): L: 0,53-0,98 mm (moyenne: 0,82 mm)
a: 31,1-46,3 (moyenne: 38,6)
b: 5-5,5 (moyenne: 5,2)
c: ?
T: 51,6-54,2 % (moyenne: 53,2 %)

1) Ingenieur-Agronome, en stage pour le compte du gouvernement mexicain.

Stylet: 23-25 μ (moyenne: 24 μ)
Spicules: au moins 25-27 μ

Allotype: L: 0,89 mm — a: 35,1 — b: 5,5 — c: ? — stylet: 24 μ — spicules: 27 μ.

Description: Habitus droit ou faiblement courbé ventralement dans la moitié postérieure du corps. Corps moyennement effilé vers l'avant, plus vers l'arrière.

Lèvres en dôme renflé, séparées du corps par une incisure nette et comportant quatre anneaux. Sclérotisation céphalique bien développée; plaque basale bombée vers l'avant. Stylet long de 23-25 μ; prorhabdion et partie postérieure de même longueur; boutons arrondis à la base et à face antérieure plane ou très légèrement convexe.

Procorpus cylindrique. Bulbe médian ovale allongé; valvules peu développées. Portion glandulaire basale très étirée, les trois glandes ne se terminant pas toujours au même niveau et parfois légèrement détachées les unes des autres. Orifice de la glande oesophagienne dorsale débouchant à 5-7 μ de la base du stylet. Intestin sans caractères particuliers.

Anneau nerveux épais, situé en-dessous du bulbe oesophagien médian. Pore excréteur à 83-136 μ de l'avant. Hemizonide plat s'étendant sur deux anneaux et situé à 3-7 anneaux en avant du pore excréteur.

Gonade très longue. Spicules fortement courbés, longs de 25-27 μ au moins, la longueur exacte ne pouvant être donnée car aucun spicule n'a pu être observé en vue latérale. Spicules épais dans leur partie proximale, puis lamellaires dans la moitié distale, l'aplatissement se faisant dans le plan sagittal; l'extrémité porte deux pointes courtes.

Cuticule annelée transversalement; anneaux larges de 1,2-1,3 μ dans la partie médiane du corps. Champ latéral non aréolé, occupant environ 1/5 du diamètre correspondant, composé de quatre lignes longitudinales parallèles non indentées, les deux lignes centrales assez rapprochées l'une de l'autre.

Queue très courte, pratiquement inexistante, arrondie, avec deux indentations (en vue ventrale ou dorsale). Phasmides non observées.

Femelles (Fig. 1, A; Fig. 2; Fig. 3).

Dimensions des kystes: (92)
longueur: 0,31-0,81 mm (moyenne: 0,571 mm)
largeur: 0,22-0,69 mm (moyenne: 0,457 mm)

Description: Les kystes mûrs de *H. oryzae* n. sp. sont de couleur brun foncé à noire. De forme assez variable, ils sont grossièrement limoniformes mais peuvent être plus ou moins sphériques, quelquefois en cornemuse. Le cône vulvaire est bien marqué, quoique moins développé que chez certaines autres espèces. Les restes de la tête ne sont pas toujours visibles; ils sont rarement situés dans le pro-

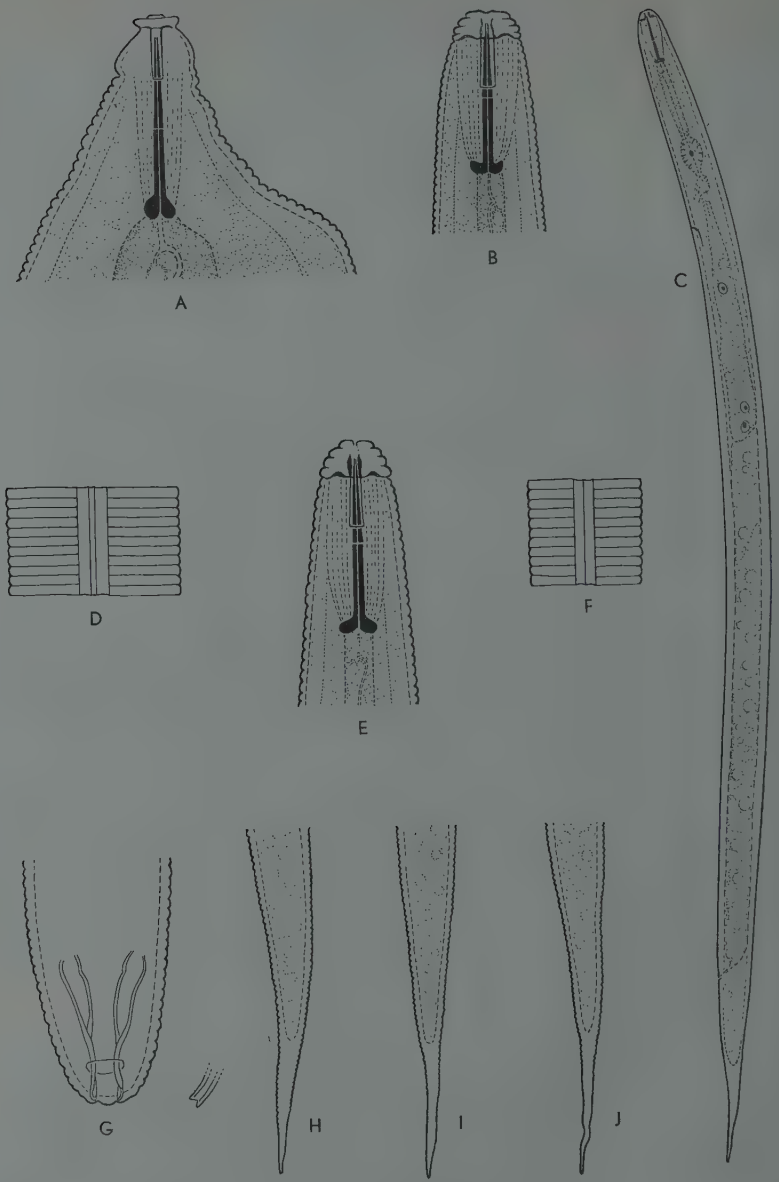

Fig. 1. — *Heterodera oryzae* n. sp. — Femelle jeune: A: partie antérieure — Mâle: D: champ latéral — E: tête — G: spicules en vue ventrale; à droite: détail de la pointe d'un spicule, en vue latérale — Juvénile au deuxième stade: B: tête — C: vue totale — F: champ latéral — H, I, J: partie postérieure.

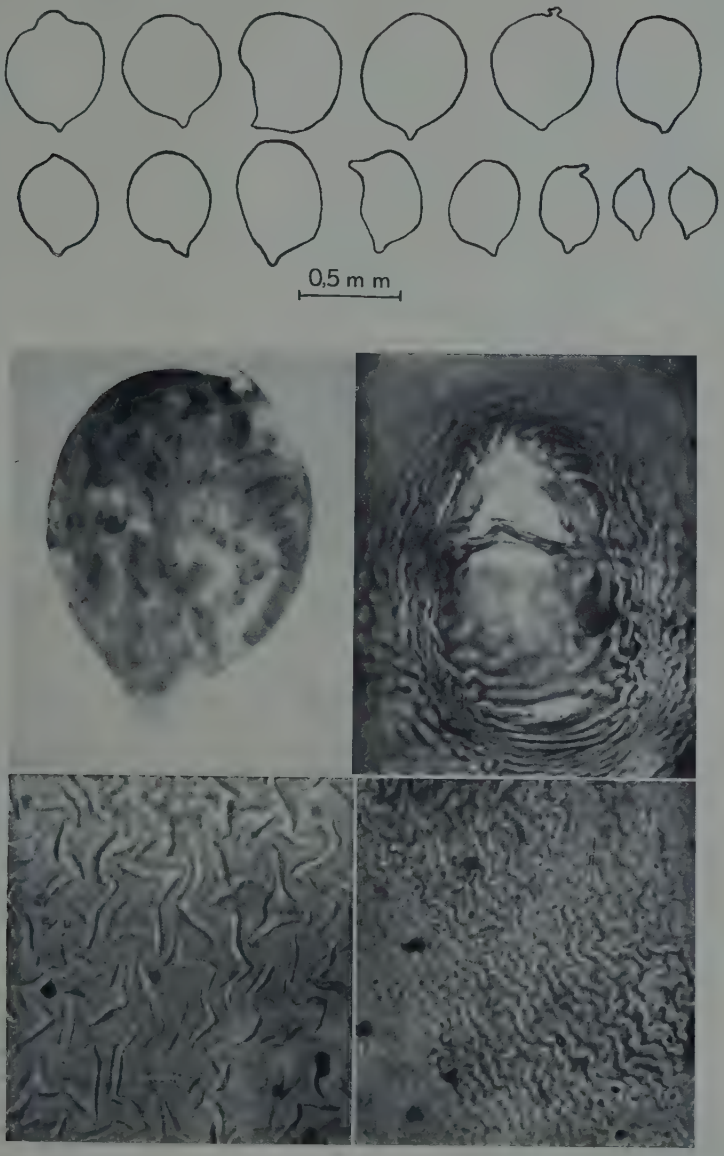

Fig. 2. — En haut: kystes — Au milieu, à gauche: jeune kyste encore transparent (coloration à la fuchsine acide); au milieu à droite: extrémité du cône vulvaire d'un kyste vue de face; en bas à gauche: ornamentation cuticulaire d'une jeune femelle; en bas à droite: ornementation cuticulaire d'un kyste, sa partie équatoriale.

longement de la vulve de telle façon que le cône vulvaire paraisse parfois presque équatorial.

Les kystes sont recouverts, rarement sur toute leur surface, par une couche subcristalline épaisse. La paroi propre du kyste est ornée de lignes en zigzags courts, sans orientation reconnaissable. Cette ornementation est surtout visible à l'avant du kyste, dans la région avoisinant les restes de la tête, et dans la portion postérieure, à la base du cône vulvaire. Au centre du kyste il est généralement impossible de découvrir la moindre trace d'ornementation; tout au plus remarque-t-on alors des séries de petites perforations, extrêmement refringentes, parfois groupées en faisceau, mais non orientées.

Vue de face l'extrémité postérieure du cône vulvaire apparaît du type ambifenestré, avec des fenestrae basses, un pont vulvaire étroit et linéaire et une fente

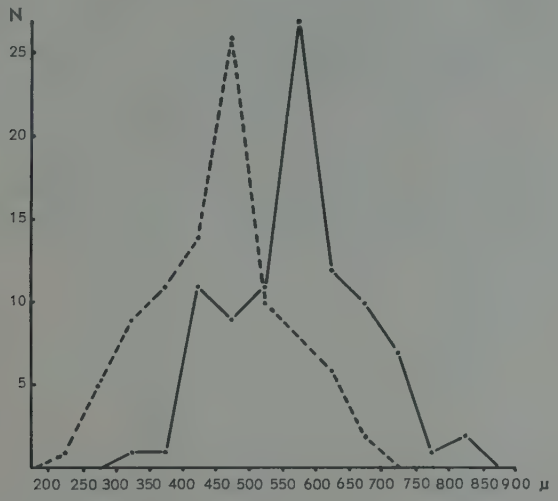

Fig. 3. — *Heterodera oryzae* n. sp. — 92 kystes — Distribution de la longueur (en trait plein) et de la largeur (en pointillé).

vulvaire de même longueur que le pont environ. Des bullae existent, moyennement développées et situées à une certaine distance en-dessous de l'ouverture vulvaire, se rapprochant plus ainsi du type "*schachtii*" que du type "*major*".

Le nombre des oeufs contenus à l'intérieur des kystes est variable (60-680 suivant la taille des kystes). Quelques kystes ont été observés qui portaient une masse d'oeufs extérieure noyée dans une gelée brunâtre et attachée à la partie postérieure du corps.

Chez les jeunes femelles encore non transformées en kyste la structure de la partie avant du corps est la suivante:

Les lèvres soudées forment un bourrelet continu, surmonté par le disque labial, assez en relief. Sclérotisation céphalique très faible. Stylet long de 28-30 μ avec

prorhabdion et partie postérieure sensiblement de même longueur; boutons basaux arrondis postérieurement et convexes antérieurement. Oesophage trapu, très développé, notamment le bulbe médian. Glande oesophagienne dorsale débouchant à 4-5 μ en-dessous de la base du stylet. Pore excréteur à 100-110 μ de l'avant. Autres structures internes non observées.

La cuticule chez ces jeunes femelles ne présente d'anneaux bien reconnaissables que dans la partie tout à fait antérieure du corps; sur certains individus ces anneaux se résolvent en points. Sur le reste du corps on distingue une ornementation en zigzags formée de lignes brisées non continues, sans aucune orientation générale reconnaissable.

Oeufs (Fig. 4).

Dimensions (88):

longueur: 90-120 μ (moyenne 104 μ)
largeur: 40-57 μ (moyenne 48 μ)
L/1: 1,8-2,6 (moyenne 2,2)

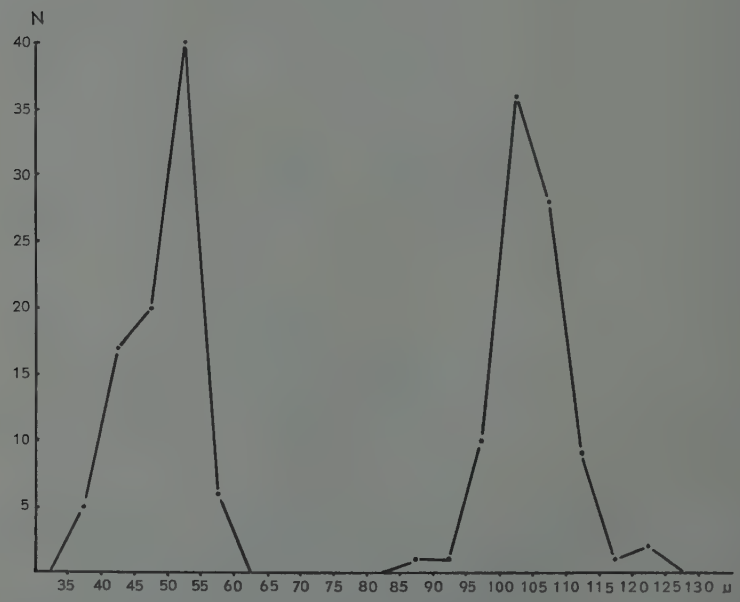

Fig. 4. — *Heterodera oryzae* n. sp. — 88 œufs — Distribution de la longueur (à droite) et de la largeur (à gauche).

La cuticule des oeufs est mince (1-2 μ), lisse, sans ornementation. La larve est repliée en 5 à l'intérieur.

Le nombre des oeufs par kyste est grossièrement lié à la dimension de ceux-ci. Voici quelques chiffres pour des kystes remplis d'oeufs:

kystes		Nombre
longueur	largeur	d'œufs
0,72 mm	0,62 mm	680
0,77 mm	0,69 mm	471
0,74 mm	0,66 mm	425
0,83 mm	0,67 mm	416
0,75 mm	0,46 mm	401
0,66 mm	0,51 mm	350
0,37 mm	0,29 mm	99
0,50 mm	0,35 mm	89
0,51 mm	0,36 mm	69
0,40 mm	0,23 mm	60

D'après les comptages et les mensurations d'oeufs effectués, le nombre d'oeufs par kyste, de même que le volume de ceux-ci n'ont aucune influence sur les dimensions des oeufs.

Juvéniles, stade 2 (Fig. 1, B, C, F, H. I. J; Fig. 5)

Dimensions: L: 0,373-0,507 mm (moyenne: 0,440 mm, n = 100)
a: 22,6-28,0 (moyenne: 24,7, n = 25)
b: 2,4-3,3 (moyenne: 2,7, n = 25)
c: 6,4-6,6, n = 2
largeur: 16,5-19,5 μ (moyenne: 17,7 μ, n = 25)
stylet: 19,5-22,0 μ (moyenne: 20,8 μ, n = 25)
partie vide de l'extrémité caudale: 35-45 μ (moyenne: 39,5 μ, n = 25)

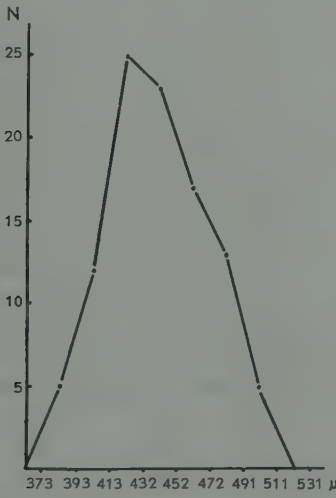

Fig. 5. — *Heterodera oryzae* n. sp. — 100 juvéniles, stade 2 — Distribution de la longueur.

Description: Habitus presque rectiligne ou très légèrement courbé ventralement (sur animaux tués par la chaleur). Corps robuste, très peu aminci vers l'avant, effilé vers l'arrière.

Lèvres hémisphériques comportant quatre anneaux et séparées du corps par une incisure fine mais nettement marquée. Sclérotisation céphalique bien développée; plaque basale bombée vers l'avant. Stylet long de 19,5-22,0 μ, le prorhabdion étant légèrement plus court que la partie postérieure (9-10 μ). Boutons basaux fins, nettement détachés, concaves antérieurement mais sans pointe.

Procorpus cylindrique, rétréci à la jonction avec le bulbe médian. Celui-ci moyennement développé; valvules peu apparentes. Portion glandulaire basale très longue, d'abord mince, puis élargie, massive, se terminant en ne recouvrant que très peu et ventralement la portion antérieure de l'intestin. Cette portion glandulaire de l'oesophage comporte trois noyaux, l'un environ au milieu, les deux autres très postérieurement. Orifice de la glande oesophagienne dorsale débouchant à 4,5-6 μ de la base du stylet. Intestin sans caractères particuliers. Anus très difficilement perceptible.

Anneau nerveux épais, situé à quelque distance en-dessous du bulbe oesophagien médian. Pore excréteur à 80-98 μ de l'avant. Hémizonide non observé.

Cuticule mince; anneaux larges de 1,2 μ au milieu du corps. Champ latéral non aréolé, large de 3-3,5 μ (1/5 à 1/6 du diamètre correspondant) et composé de trois lignes longitudinales non crénelées.

Queue conique, longuement effilée; chez les deux seuls juvéniles où l'anus a pu être observé elle mesure 67 et 69 μ. La queue comporte à sa partie postérieure une portion vide, hyaline, assez importante (35 à 45 μ). Phasmides non observées.

Holotype: kyste; tube n° 486.

Allotype: mâle; n° 1, lame 902-CI-435.

Pédotype: juvénile, 2ème stade; n° 7, lame 893-CI-488.

Tous ces types sont déposés dans la collection du Laboratoire de Nématologie — I.D.E.R.T. — Abidjan (Côte d'Ivoire).

Hôte type: racines; *Oryza sativa* L., var. Moroberekan.

Lieu type: village de Bokakouamékro, 40 km à l'Est de Bouaké (Côte d'Ivoire).

Diagnose: *Heterodera oryzae* n. sp. peut aisément se caractériser: il s'agit en effet de la seule espèce du genre *Heterodera* à posséder à la fois des kystes avec cône vulvaire et des juvéniles chez lesquels la portion hyaline de la queue est nettement plus longue que le stylet (ici presque deux fois). Ce dernier caractère joint à l'aspect extrêmement effilé de la partie postérieure du corps de ces mêmes juvéniles sont les meilleurs moyens d'identification pour cette espèce, les kystes n'ayant aucun caractère vraiment particulier.

Heterodera oryzae n. sp. se distingue tout à fait aisément des deux autres espèces du genre parasitant les Graminées: *H. major* O. Schmidt, 1930 et *H. punctata* Thorne, 1928. De la première elle diffère par ses larves nettement plus petites: 440 μ en moyenne contre 575 μ (Franklin, 1951), avec un stylet également plus petit (21 μ contre 26 μ) et une portion hyaline de la queue beaucoup plus importante; de plus si l'on observe chez *H. major* des masses gélatineuses attachées à certains kystes, elles ne contiennent pas d'oeufs. De *H. punctata,* qui possède également des juvéniles à queue effilée et portion hyaline plus longue que le stylet, *H. oryzae* n. sp. diffère essentiellement par la forme du kyste: les

kystes de *H. punctata* ne possèdent pas de cône vulvaire et sont du type bifenestré, alors que ceux de *H. oryzae* n. sp. ont un cône vulvaire et sont du type ambifenestré.

La forme en citron des kystes, la présence d'un cône vulvaire, l'ornementation des kystes constituée de lignes en zigzags non orientés transversalement, la présence de bullae, le fait que des oeufs puissent être émis en dehors des kystes, permettent de classer *H. oryzae* n. sp. dans le groupe *schachtii*.

Peu de kystes ont été rencontrés dans le sol et la plupart d'entre eux étaient vides d'oeufs ou n'en contenaient qu'un petit nombre. D'assez nombreux kystes, même ceux prélevés directement sur ou dans les racines, sont parasités intérieurement par plusieurs champignons dont un Basidiomycète, mais ces champignons qui tapissent parfois entièrement la face intérieure de la paroi du kyste n'ont jamais été observés à l'intérieur des oeufs eux-mêmes.

De nombreux juvéniles extraits des racines à l'asperseur étaient extérieurement parasités par *Dubosqia penetrans* Thorne, 1940.

Nous remercions très vivement pour sa collaboration M. B. Souchaud qui a effectué les photographies et la plupart des montages.

SUMMARY

The authors describe *Heterodera oryzae* n. sp., parasitic on lowland temperate rice, variety Morobérékan, grown at Bokakouamékro, near Bouaké (Ivory Coast). This species of the *schachtii* group is characterized by its dark brown to black cysts, measuring 0.31-0.81 × 0.22-0.69 mm (mean: 0.57 × 0.45 mm); these cysts are lemon-shaped, with a subcrystalline layer and ornamentation in zig-zag lines but with no trace of regular transverse markings; the vulval cone is prominent and the cone top is of the ambifenestrate type with bullae. The second stage larvae measure between 0.37 and 0.50 mm (mean: 0.44 mm); their stylet is of 19.5-22 μ long; these larvae are characterized mainly by their lateral field with only three lines and by the acutely pointed tail with a hyaline portion much longer than the stylet (35-45 μ). Males are rare and resemble those of *H. schachtii*.

RESUMEN

La descripción dada por los autores corresponde al *Heterodera oryzae*, nueva especie encontrada parasitando un cultivo de arroz de temporal de la variedad Moroberekan, sembrado en el poblado de Bokakouamekro, cerca de Bouaké (Costa de Marfil). Esta especie del grupo *schachtii* tiene las caracteristicas siguientes:

Quistes en forma de limón, de color café obscuro a negro, cuyas dimensiones oscilan entre 0,31-0,81 × 0,22-0,69 mm (promedio 0,57 × 0,45 mm), con ornamentaciones en zig-zag sin órden transversal definido y cubiertos parcialmente por una capa semicristalina. El cono vulvar es prominente con remate del tipo ambifenestrado con „bullae". La longitud del segundo estado larval oscila entre 0,37 y 0,50 mm con un promedio de 0,44 mm y un estilete de 19,5 a 22 μ de largo. Se caracterizan principalmente por presentar un campo lateral de únicamente tres lineas y hacia la parte caudal una porción hialina de mayor longitud que le estilete (33-45 μ). Los machos son raros y éstos se asemejan a los del *H. schachtii*.

BIBLIOGRAPHIE

Cooper, B. A. (1955). A preliminary key to British species of *Heterodera* for use in soil examination — in *Soil Zoology*, Ed. D. K. McE Kevan, London, Butterworths, p. 269-280.

—— (1955). Mounting technique for identification of *Heterodera* eelworm cysts. *Ibid.* 419-420.

Franklin, M. T. (1951). *The cyst forming species of Heterodera.* Farnham Royal, Comm. Agric. Bur., 148 pp.

Hesling, J. J. (1959). The identification of *Heterodera* cysts — in *Plant Nematology, Techn. Bull. no. 7, Min. Agric.*, Ed. J. F. Southey, England, 64-69.

Luc, M. (1961) — *Heterodera* spp. on tropical crops in Africa. *Nature. Lond.* 191. In the press.

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THE EFFECT OF SOIL TEMPERATURE ON THE SURVIVAL OF THE ROOT-KNOT NEMATODES *MELOIDOGYNE JAVANICA* AND *M. HAPLA* [1])

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Plant roots infected with the root-knot nematode, *M. javanica,* were obtained from Southern Rhodesia, North Carolina and Georgia. These widely separated areas represent a diversity of soil temperature and soil moisture conditions. Stock cultures of each of the three populations, S.R., N.C. and Ga. were established from single egg-masses. Egg-masses of the three *M. javanica* populations and of *M. hapla* were exposed to various soil temperatures in the laboratory and to over-wintering in the field. Viability of eggs after treatment was measured by the bioassay method.

At a soil temperature of —2° C eggs of *M. hapla* can survive for a longer period than those of *M. javanica.* Of these populations S.R. has the least tolerance, N.C. has the greatest, and Ga. is intermediate. A longer period is required to kill eggs of all populations in dry soil than in damp soil. However, at 36° C and 40° C eggs are killed more rapidly in dry soil. The response of *M. javanica* and *M. hapla* to these high temperatures is the reverse of that found at —2° C, eggs of the latter are less tolerant than those of *M. javanica.* The N.C. eggs are least able to withstand high temperatures, those of S.R. are most tolerant and Ga. is intermediate. Individuals within each of the four populations exhibit increased tolerance to —2° C and others to 36° C. Tolerance to —2° C is not associated with response to high temperatures.

The ability of *M. hapla* eggs to over-winter in the field is marked. Differences between the *M. javanica* populations are pronounced. Egg-viability of S.R. is greatly reduced after 98 days and none survived 139 days or longer. The N.C. eggs show only a moderate reduction in numbers after 98 days and some remain viable for 208 days. The Ga. population is intermediate, few eggs remaining viable after 139 days in the field. Differences of the degree of tolerance of the *M. javanica* populations may to some extent be due to conditions under which the three populations have evolved.

Root-knot nematodes of the genus *Meloidogyne* Goeldi are worldwide in distribution and are important parasites of a wide range of economic plants. Much work on various phases of the taxonomy, life history, biology and control of these nematodes has been done during the past century and the literature on these subjects is extensive. However, knowledge of morphological and physiological variations among populations is comparatively recent. Hence, much of the early work is difficult to interpret and, in the light of present knowledge, the need for further study of many aspects of the root-knot nematode problem is recognized.

The response of root-knot nematodes to various environmental factors has

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been studied extensively because of its bearing upon geographical distribution, population development and survival in relation to the epidemiology and control of root-knot. Most species of *Meloidogyne* are important plant pathogens in tropical and semi-tropical regions and in temperate areas having relatively mild winters. Temperature, therefore, is one of the most important factors that governs the occurrence and severity of root knot in various parts of the world.

Much of the work on the effect of temperature upon root-knot nematodes was done during the period when these organisms were regarded as a single species. Since Chitwood's (1949) taxonomic revision of root-knot nematodes, several temperature studies have been made with populations of certain species and subspecies of *Meloidogyne* (Brown 1955, Santmyer 1955, van der Linde 1956, Thomason 1957, Walker 1960). Dropkin (1957) and Taylor & Buhrer (1958) have shown that different species of *Meloidogyne* have different climatic ranges.

The existence of races or strains within certain species of *Meloidogyne* that vary in their host-parasite relationships has been reported by Martin (1954), Sasser & Nusbaum (1955), Powell (1957) Colbran (1958), Dropkin (1959), Riggs & Winstead (1959), Goplen, Stanford & Allen (1959), and Triantaphyllou & Sasser (1960). No doubt strains also exist that show other kinds of physiological variations. Populations of various species of *Meloidogyne,* under the dynamic influences of different climatic and soil conditions as well as different systems of land management and crop culture, are subjected to various selection pressures which may affect the composition of strains among the populations of a single species.

The present study compares the temperature responses of three populations of *Meloidogyne javanica* (Treub) from different places. One population of *M. hapla* Chitwood was included for comparison with the *M. javanica* cultures. The study was divided into three parts: (1) the tolerance of eggs (in masses) to low and high temperatures in damp and dry soil; (2) the ability of eggs to over-winter in the field; and (3) the homogeniety of the progeny of each population in response to high and low temperatures.

MATERIALS AND METHODS

Root-knot nematode cultures

Meloidogyne javanica was obtained from Southern Rhodesia (Africa) and from North Carolina and Georgia (U.S.A.). *M. hapla* was obtained from North Carolina. Southern Rhodesia is an area having predominantly high temperatures. North Carolina has much lower minimum temperatures and slightly lower maximum temperatures. Temperature ranges in Georgia are intermediate between those cited above.

Cultures were increased on greenhouse grown tomato plants (Rutgers var.). When populations of mature females with well developed egg masses were produced, single egg-mass cultures of each population were established on potted

tomato plants. In each case, identification was confirmed by examination of perineal patterns (Taylor, Dropkin & Martin, 1955). Originally, several single egg-mass cultures of each population were established but only one of each was chosen for the temperature experiments. The populations of *M. javanica* are designated as follows: N.C. — from North Carolina; Ga. — from Georgia; and S.R. — from Southern Rhodesia.

Greenhouse procedures were standardized so that plants of known age and inoculum from cultures of uniform age would be available when needed. The soil used in greenhouse and laboratory was prepared by sieving and mixing four parts of steamed, coarse sand with three parts of steamed, loamy sand. This mixture had a moisture holding capacity of 9.6 percent at 60 cm tension. Egg masses from 60-day-old cultures were used as inoculum in all experiments. They were transferred immediately after collection to the soil in an experimental container.

Bioassay

Survival of eggs was determined by the bioassay method (Godfrey, 1934). After completion of the treatment, each sample of eggs in soil was transferred to a 15-cm pot half filled with soil, a tomato seedling was transplanted and additional soil was added to fill the pot. After 35 days' growth, long enough for primary galls to develop yet not long enough for secondary galls to form, the indicator plants were removed. The soil was carefully washed from the roots which were spread out in a small quantity of water in a flat bottomed vessel and examined for gall formation. The extent of galling, a measure of egg survival, was rated on a 0 to 8 scale with the following index values:

Infection class	*Index value*	*Description of index value*
0	0	Free from galls.
1	1	Trace, less than 5 galls.
2	5	Very slight, trace to 25 galls.
3	10	Slight, 26 to 100 galls.
4	25	Moderate, galls numerous, mostly discrete.
5	50	Moderately heavy, galls numerous, many coalesced.
6	75	Heavy, galls very numerous, mostly coalesced, root growth slightly retarded.
7	90	Very heavy, mass invasion, slight root growth.
8	100	Extremely heavy, mass invasion, no root development.

Experimental data are based upon mean index values. When shown in line graphs, the index value of the non-treated control for each population is plotted on the ordinate.

Soil temperature studies

Thermostatically controlled temperature chambers fitted with thermometers to show soil temperatures, were used. The chambers holding all of the soil sample

containers were set up about 48 hrs before the beginning of each test so that the soil could become stabilized at the desired temperature before egg-masses were introduced.

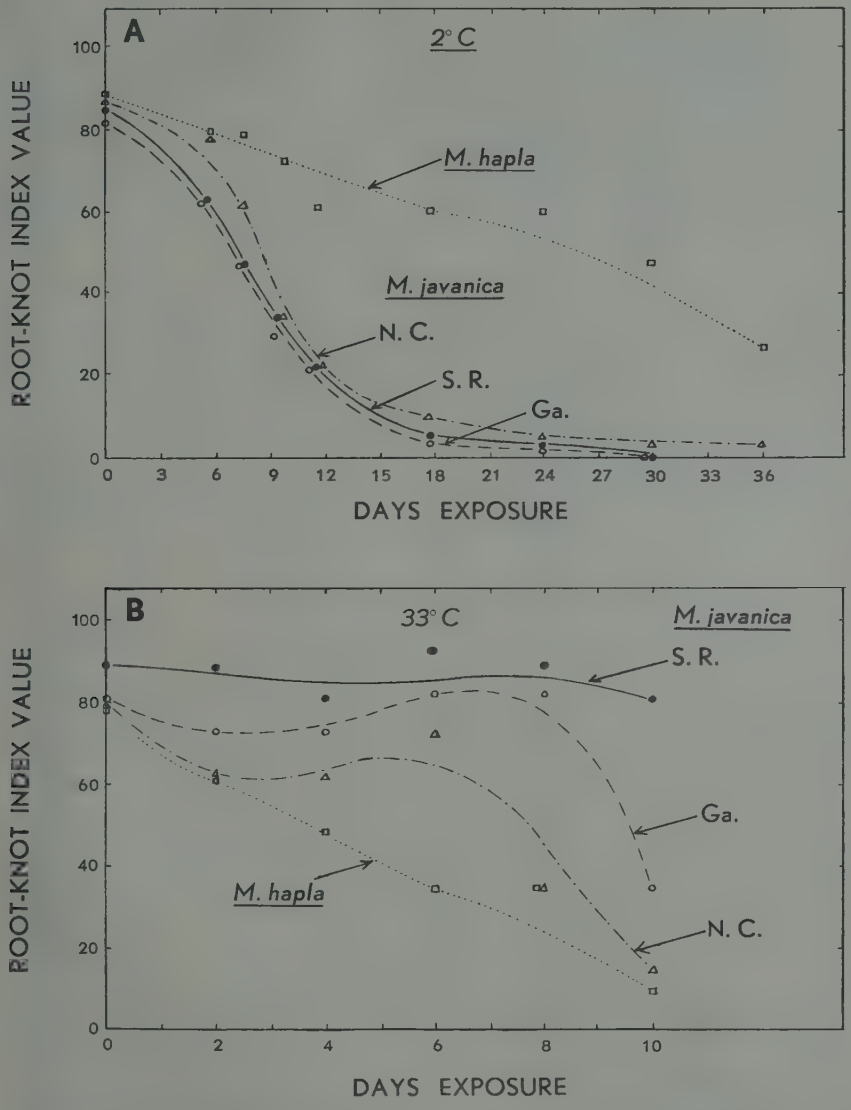

Figure 1. The survival of root-knot egg-masses to soil temperatures of 2° C (A) and 33° C (B) in damp soil determined by root-knot indices of indicator plants.

Twenty egg-masses of each population were used for every replicate and were placed in rigid plastic dishes 7 cm in diameter and 1.5 cm in depth approximately at the centre of the soil mass. There were two replications of each treatment. In every experiment, 20 egg-masses of each population were set up immediately for bioassay to check egg viability.

Temperature exposures were made in both dry and damp soil. In the former, the soil was air dried to a moisture content of 1.4 percent. In the latter, the moisture content was raised to 6.1 percent by the addition of distilled water. This moisture level was maintained throughout the exposure period by providing a high relative humidity in the chambers. This was accomplished by placing water in shallow pans located in the chambers at a level below that of the soil containers. These pans were replenished when necessary with water at the same temperature as that of the chamber.

Over-wintering of egg-masses in the field

15-cm pots of soil, infested with 100 egg-masses at a depth of 10 cm, were set in field plots so that the tops of the pots were at ground level and kept free from weeds. The field soil was a clay loam, free from root-knot nematodes. Duplicate pots, individually infested with the four test populations, were set out at approximately 38-day intervals between August 1958 and January 1959. All pots were removed in April 1959, having been exposed for 250, 208, 173, 139 and 98 days, respectively. Survival was estimated by the bioassay method as previously described.

RESULTS

Laboratory studies

Survival of root-knot nematode eggs (in masses) in damp soil was tested at 2° and 33° C. The results are presented in Figure 1. Eggs of *M. hapla* were more tolerant to 2° C and less tolerant to 33° C than those of *M. javanica.* The S.R. eggs were unaffected by 10 days exposure to 33° C, whereas the viability of Ga. and N.C. eggs was reduced markedly. The N.C. eggs were somewhat more tolerant to 2° C and less tolerant to 33° C than those of either S.R. or Ga. More extreme temperatures than 2° and 33° C and relatively short exposure periods would be needed to differentiate survival thresholds of the three populations. Accordingly, a second experiment was made at —2°, 36° and 40° C each at two soil moisture levels of 6.1 percent (damp) and 1.4 percent (dry).

The results of the experiment at —2° C are shown in Figure 2. The differences between *M. javanica* and *M. hapla* eggs were as pronounced as at +2° C but the exposure periods required to reach the survival thresholds were much less. The maximum survival of N.C. eggs was 6 days at —2° C compared with more than 36 days at +2° C. In damp soil at —2° C loss of viability of S.R. eggs was rapid. The root-knot index value being 5.5 after 6 hrs. compared with 75 hrs for the untreated control. The S.R. eggs were the least tolerant of exposure to —2° C

in damp soil for 6 hrs as shown by very slight galling of indicator plant roots. In contrast, indicator plants inoculated with either Ga. or N.C. populations thus

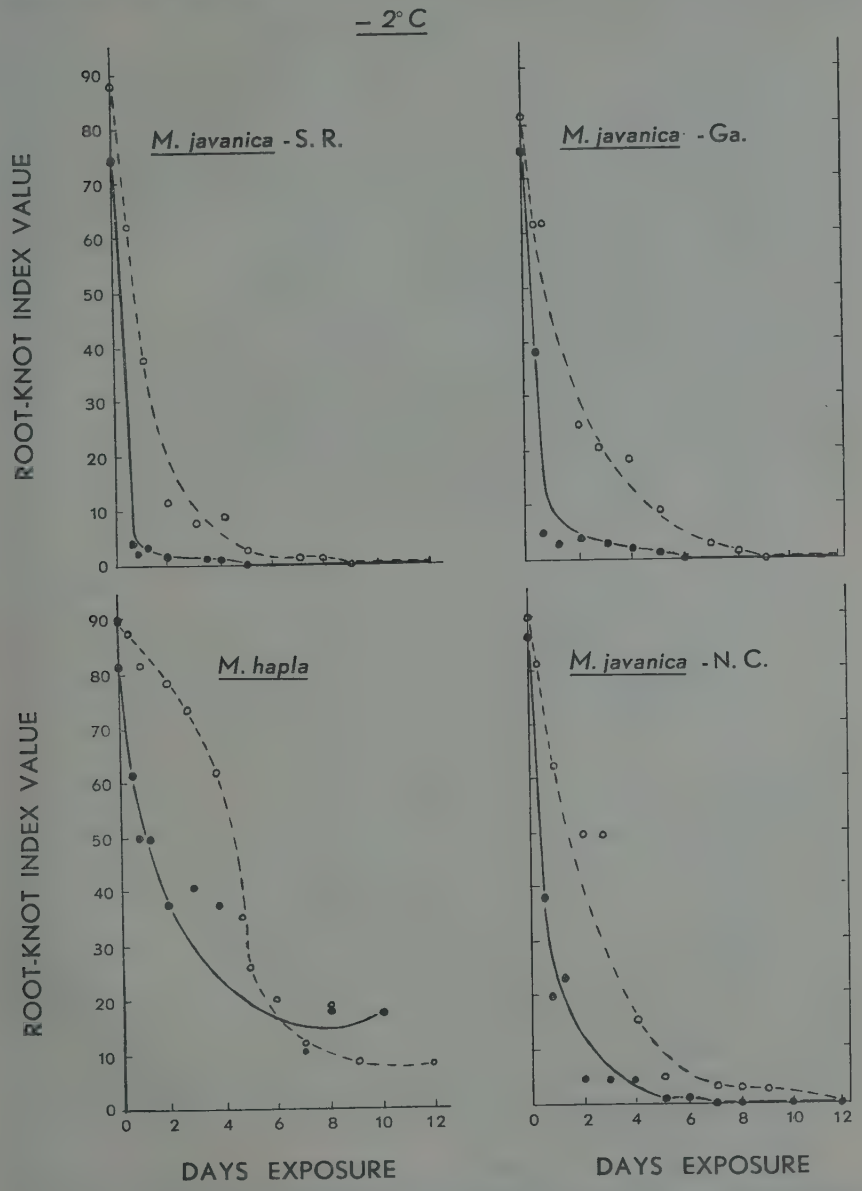

Figure 2. The survival of egg-masses at a soil temperature of —2° C in air-dried and damp soil determined by root-knot indices of indicator plants.

exposed were moderately galled, indicating higher tolerance. *M. hapla* eggs were least affected and produced moderate to heavy infection of indicator plants.

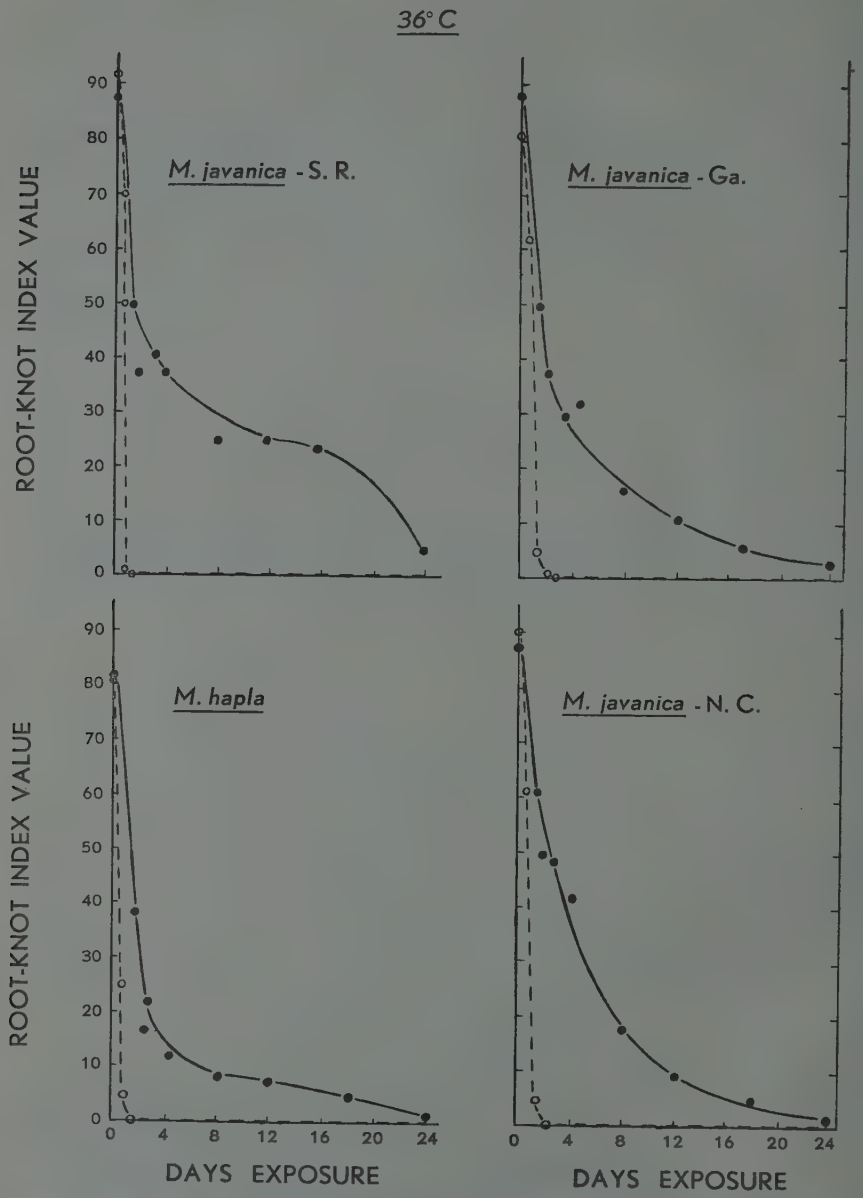

Figure 3. The survival of egg-masses at 36° C in air-dried and damp soil determined by root-knot indices of indicator plants.

In dry soil a longer exposure was necessary to kill eggs than in damp at —2° C (Figure 2). There was little difference in the exposure required to obtain a zero index value for *M. javanica,* all populations being non-viable after 12 days. Even so, the N.C. eggs were more tolerant than the S.R. and Ga. eggs. The S.R. population was nearly dead after 3 days, whereas the Ga. and N.C. populations produced moderate and heavy root galling upon their respective indicator plants. The viability of *M. hapla* eggs in dry soil at —2° C decreased more slowly until the fifth day after which rapid loss occurred. The longest exposures used, 10 and 12 days, were not sufficient to kill all *M. hapla* eggs.

The exposure required to render the eggs non-viable at 36° C differed markedly in damp and dry soil (Figure 3). Some eggs of all populations were able to survive an exposure of 24 days in damp soil, but none survived after 12 hrs in dry soil. The results agree with the first experiment which showed the S.R. eggs could withstand exposure to 33° C better than the others. Root-knot index values of S.R. populations in damp soil at 36° C were above 20 after 16 days whereas those of Ga. and N.C. dropped below 20 after only 8 days. *M. hapla* populations were even more sensitive, the index value falling to about 10 on the fourth day. Decrease in egg viability was extremely rapid in dry soil at 36° C. After 6 hrs exposure the root-knot index for the S.R. population had dropped from a control value of 82.5 to 50.0. During this same period, index values of Ga. and N.C. populations had dropped to 5.0.

There were marked differences between the effects of an 18-hr exposure in damp soil (Figure 4A) and a 6-hr exposure in dry soil (Figure 4B) at 36° C. The viability of the eggs of all populations was less affected in damp than in dry soil although the exposure period was longer in the former. This soil moisture effect, as well as the differential responses of the populations, was the reverse of that found in experiments at —2° C (Figure 2).

Results in dry soil at 40° C were similar to those at 36° C. In damp soil, however, 40° C reduced egg viability more rapidly than 36° C. The minimum exposure that gave a zero root-knot index for *M. javanica* ranged from 1 to 4 days compared with 24 days at 36° C. Eggs in dry soil at 40° C were killed after 3 to 12 hrs which corresponded closely to the results at 36° C. The S.R. populations tolerated longer exposures in damp soil at 40° C than Ga. and N.C. populations and *M. javanica* was more tolerant than *M. hapla.* Galling of indicator plant roots was similar for the Ga. and *M. hapla* populations after 6 hrs, but that of the latter was negligible after 12 hrs while that of the former remained high. At the same temperature in dry soil, but after only 3 hrs exposure, the amount of galling caused by *M. javanica* populations was heavy whereas that of *M. hapla* populations was greatly reduced. These findings agree with the results obtained at 33° and 36° C.

More experiments were done to determine whether individuals within each population are more tolerant than others to high or low temperatures and whether such individuals could be selected by exposure of the populations to temperature

extremes. Egg-masses were removed individually from galls on indicator plant roots where very low index values showed that most individuals had succumbed. These selections were bred on tomato plants and then compared with the original populations by exposing 20 egg-masses of each to —2° C or 36° C in damp soil. Exposures were chosen which would reduce survival to a low level.

TABLE I

*Tolerance to —2° C by single egg-mass cultures, selected from individuals that had survived exposure to this temperature * compared with that of their respective stock cultures*

Population	Length of exposure in hrs.	Mean root-knot index	
		Stock culture	Selected culture *
S.R.	6	12	38
Ga.	6	26	41
N.C.	12	26	32
M. hapla	240	8	38

* Exposure time at —2° C (damp soil) from which selected egg-masses were obtained: S.R. — 1 day; Ga. — 1 day; N.C. — 3 days; and *M. hapla* — 10 days.

TABLE II

*Tolerance to 36° C of single egg-mass cultures, selected from individuals that had survived exposure to this temperature * compared with that of their respective stock cultures*

Population	Length of exposure in hrs.	Mean root-knot index	
		Stock culture	Selected culture *
S.R.	192	33	67
Ga.	192	18	33
N.C.	192	25	42
M. hapla	84	18	25

* Exposure time at 36° C (damp soil) from which selected egg-masses were obtained: S.R. — 24 days; Ga. — 24 days; N.C. — 18 days; and *M. hapla* — 12 days.

The results, (Tables I and II), show that progenies of selected individuals within each population differ from the stock cultures from which they were derived in tolerance of temperature extremes. Progeny from individuals that survived —2° C were more tolerant than their respective stock cultures when tested at this temperature. Similarly, progeny from individuals that had survived exposure at 36° C, tolerated treatment at this temperature better than their respective stock

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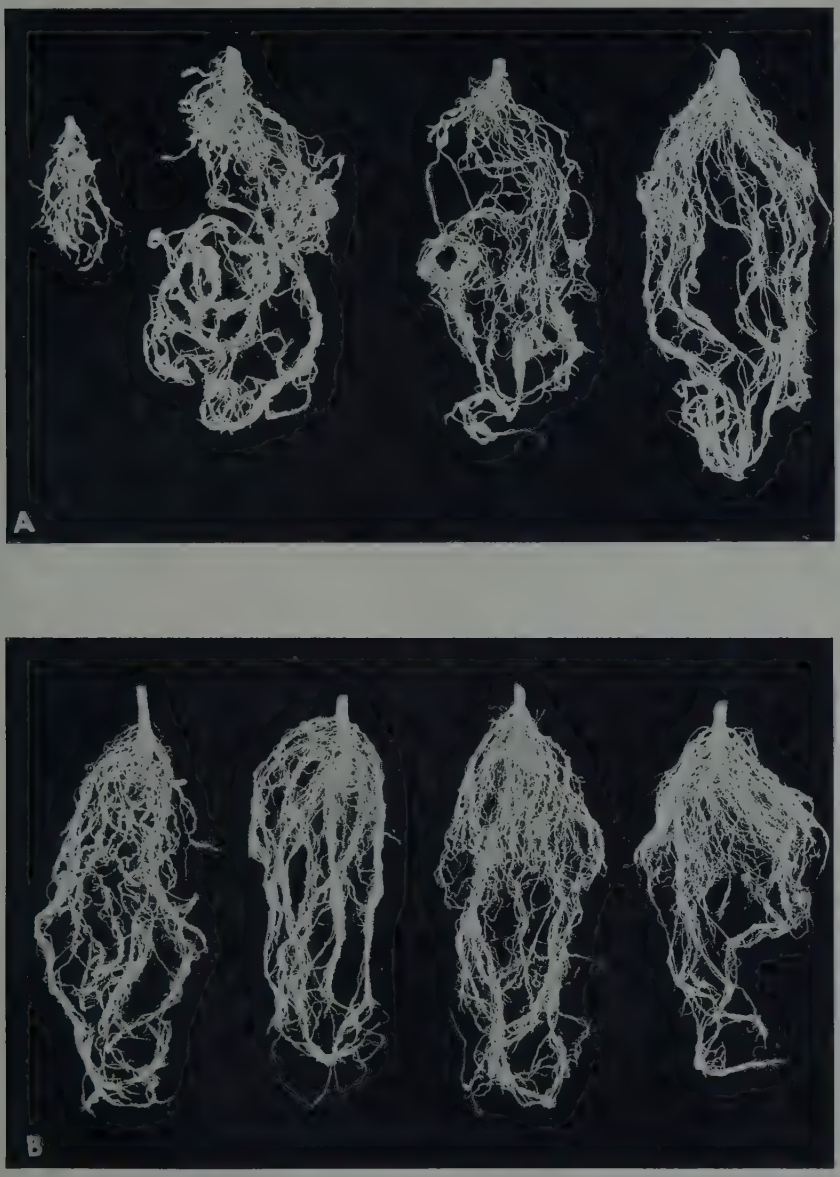

Figure 4. Root-knot gall development in tomato roots grown in soil inoculated with egg-masses previously exposed to a soil temperature of 36° C for 18 hrs in damp soil (A) and 6 hrs in dry soil (B). From left to right — root systems from soil inoculated with egg-masses of *M. javanica* populations S.R., Ga., and N.C., and *M. hapla*.

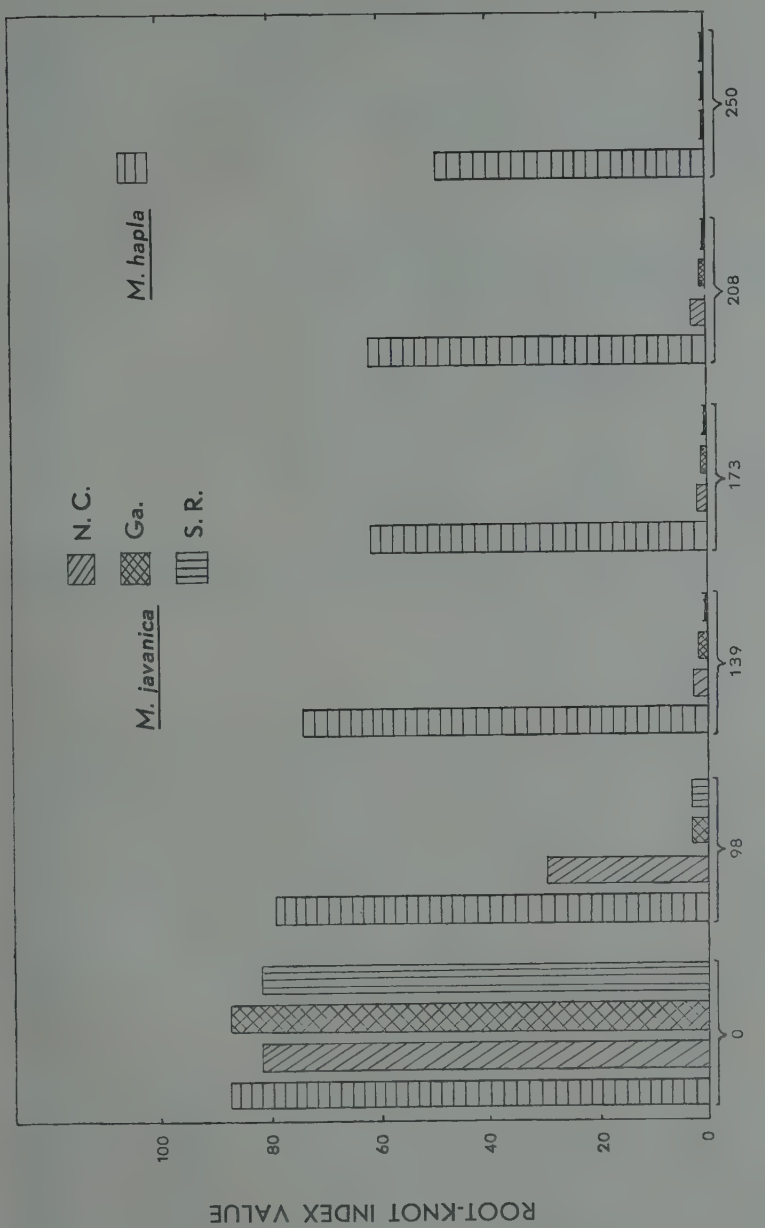

Figure 5. Survival of egg-masses to over winter in the field determined by root-knot indices of indicator plants.

cultures. Untreated controls of both selected and original stock cultures of all four populations gave index values ranging from 82.5 to 95.0

Progeny from individuals that showed increased tolerance to —2° C tested at 36° C responded in a manner similar to their respective stock cultures (Table III). The indices for the selections and stock cultures within each population showed no appreciable differences which suggests that tolerance to —2° C is not associated with high temperature response.

TABLE III

Tolerance to 36° C by single egg-mass cultures, selected from individuals that had survived exposure to —2°C compared with that of their respective stock cultures

Population	Length of exposure in hrs.	Mean root-knot index	
		Stock culture	Selected culture *
S.R.	192	31	31
Ga.	192	38	45
N.C.	192	42	31
M. hapla	84	9	9

* Exposure time at —2° C (damp soil) from which selected egg-masses were obtained: S.R. — 1 day; Ga. — 1 day; N.C. — 3 days, and *M. hapla* — 10 days.

Over-wintering of egg-masses in the field

Soil temperatures in the field at 10 cm ranged from 31° C in late August to 0° C in late January but the soil was frozen at this depth for short periods only.

Results of the field experiment (Figure 5) support those obtained in the laboratory. The ability of *M. hapla* eggs to overwinter was striking. Many *M. hapla* eggs were still viable after 250 days exposure, whereas those of the three *M. javanica* populations had succumbed. The N.C. population retained appreciable viability after 98 days exposure and a few eggs survived for 208 days. Survival after 98 days was much lower for the Ga. than the N.C. population. Beyond that point, however, both populations had low index values. The S.R. population was the least tolerant. A few individuals survived for 98 days, but none survived for as long as 139 days.

DISCUSSION

Egg masses were used to determine the ability of root-knot eggs to survive extremes of soil temperature because the eggs in the protective, gelatinous matrix may be more tolerant to adverse conditions than free larvae or eggs (Godfrey & Morita, 1929). The inoculum level of 20 egg-masses per container used in the laboratory experiments was satisfactory; control plants consistently had an index of 75 to 95. The inoculum level was raised to 100 egg-masses for the over-wintering experiments; this also gave adequate index values for comparing treatment responses of

the various populations. The 0 to 8 scale of rating also proved satisfactory and the ratings of duplicates within each treatment were usually the same and never differed by more than one class.

Because earlier work on the effect of temperature upon the root-knot nematodes was conducted before revision of the genus, comparisons of our results with those of earlier workers cannot be made. Moreover, earlier work was mainly observational from geographical distribution and records of field survival (Neal, 1889; Atkinson, 1889; Bessey, 1911). Experiments made under controlled conditions mainly concerned development and reproduction rather than egg survival. Thermal death points have been determined for larvae or eggs in water (Hoshino & Godfrey, 1933; Tyler, 1933 a & b). Santmyer (1955) found that the temperatures required to kill all eggs of *M. incognita acrita* after exposure for 1, 2, 3, 4 and 5 hrs were 46°, 45°, 44°, 43°, and 42° C, respectively. Thomason (1957) and Walker (1960) reported variable responses to temperature between *Meloidogyne* spp.

Over-wintering of egg masses of *M. hapla* and the three *M. javanica* populations in the field gave results similar to those in the laboratory. Cunningham (1936) found that winter did not materially reduce root-knot nematode infestations on Long Island, New York. *M. hapla* may have been the species studied. In England, Franklin (1937) showed that *Meloidogyne* sp. could survive 16 months out-of-doors in the absence of the host and could withstand some freezing. Kincaid (1946) found that root-knot nematodes raised in the southern states of the U.S.A. failed to survive the winter in Indiana and Wisconsin. He stated that the contradictory findings from his work and that of Cunningham might be explained by the existence of physiological races behaving differently to low temperatures. The nematodes of Kincaid were probably of some species other than *M. hapla*. Brown (1955) showed that root-knot nematodes found in greenhouse soils caused large galls without root proliferation in roots of susceptible plants. Galls from infected plants grown out-of-doors were small with much root proliferation and were caused by *M. hapla* which the author thought native to Great Britain. *M. hapla* occurs more frequently north of the latitude of Washington, D.C., than any other species, while *M. javanica* occurs in the warmer southern and south-western states (Taylor & Buhrer, 1958).

Variation in response to temperature between three populations of *M. javanica* has been demonstrated and it has been shown that the responses of these populations are related to their places of origin. The implication is that each population had been influenced by the range of temperature that characterize its natural environment. Climatic adaptation has been demonstrated in other organisms. Sparck (1936) showed that there is a correlation between water temperature and distribution of species of lamellibranches and suggested that locally different races exist within the same species. Scholander, Flagg, Walters & Irving (1953) found temperature adaptations in arctic and tropical poikilotherms.

Evidence is also presented that variation in tolerance to either high or low temperatures exists among individuals of a single population and that after exposure

to near-lethal temperatures the survivors transmit increased tolerance to their offspring. Physiological variations therefore exist within a population derived from a single egg-mass upon which environmental factors may exert selection pressures and cause evolutionary change. Whether selected populations would maintain increased tolerance in succeeding generations or whether the tolerance limits could be increased still further by continued selection, has not been determined. Knowledge of the extreme limits for such variations within species would be valuable in determining the survival potential of a species in a particular region.

This may be of importance in control by the use of resistant varieties. The tobacco variety, N.C. 95, has high resistance to *M. incognita* (Kofoid & White), the predominant species present in North Carolina, but is susceptible to *M. javanica* (Moore, Powell & Gwynn, 1961). Although *M. javanica* occurs in North Carolina, it is sparsely distributed. Our work and that of Powell (1960) shows that populations of *M. javanica* have limited ability to survive the low temperatures of the winter months. But, if the range of variability should permit a build-up of populations more tolerant to low temperature than those now in existence, this species might become an important parasite of tobacco in North Carolina and nullify the use of resistant varieties. The selection of forms capable of breaking resistance is another way in which control might break down: resistance breaking biotypes of the potato root eelworm (*Heterodera rostochiensis* Woll.) occur in Europe. This example illustrates the importance of ecological studies with *Meloidogyne* spp. Such knowledge is basic to further advances in the epidemiology and control of root knot in regions where this disease is a serious problem.

ZUSAMMENFASSUNG

Einfluss der Bodentemperatur auf das Überleben der Wurzelgallennematoden Meloidogyne javanica *und* M. hapla

Für diese Untersuchung wurden Pflanzen aus Süd-Rhodesien (S.R.), Nord Carolina (N.C.) und Georgia (Ga.) benutzt, die von Wurzelgallen-Nematoden befallen waren. Die geographisch weit voneinander entfernten Gegenden vertreten wegen ihrer klimatischen Unterschiede verschiedene Bodentemperatur- und Bodenfeuchtigkeitsbedingungen. Von je einer Pflanze aus S.R., N.C. und Ga. wurde eine Eiermasse dazu benutzt, um je eine Stammkultur zu züchten. Eiermassen der drei *M. javanica*-Kulturen und der *M. hapla*-Kultur wurden verschiedenen Bodentemperaturen unter kontrollierten Bedingungen und natürlicher Überwinterung im Freien unterworfen. Nach der Behandlung wurde die Lebensfähigkeit der Eier durch die Bioassay-Methode gemessen.

Bei einer Bodentemperatur von —2° C leben die Eier von *M. hapla* länger als die von *M. javanica.* Von diesen zeigt die S.R.-Kultur die geringste Widerstandsfähigkeit, die N.C.- die grösste, die Ga.-Kultur ist dazwischenliegend. Im trockenem Boden ist bei dieser Temperatur eine längere Zeitspanne erforderlich, um die Eier aller vier Kulturen zu töten als im feuchten Boden. Jedoch werden die Eier bei Temperaturen von 36° C und 40° C im trockenen Boden schneller getötet. Die Reaktion der *M. javanica-* und *M. hapla*-Kulturen auf diese hohen Bodentemperaturen ist den Ergebnissen bei —2° C entgegengesetzt; Eier der letzteren Kultur sind weniger widerstandsfähig als die der *M. javanica*-Kultur. Die Eier der N.C.-Kultur können am wenigsten hohe Temperaturen vertragen; die S.R.-Kulturen sind am widerstandsfähigsten und die Ga.-Kulturen liegen dazwischen.

Einzelne Larven innerhalb aller vier Kulturen zeigen eine erhöhte Widerstandsfähigkeit gegenüber der Reaktion der Stammkulturen bei diesen Temperaturen. Widerstandsfähigkeit bei —2° C ist jedoch nicht mit Reaktion gegenüber hohen Temperaturen verbunden.

Die Fähigkeit der *M. hapla*-Eier zu überwintern ist sehr ausgeprägt. Unterschiede zwischen den drei *M. javanica*-Kulturen treten scharf hervor. Nach 98 Tagen Aussetzung einer Wintertemperatur geht die Lebensfähigkeit der S.R.-Eier stark zurück und keines lebt 139 Tage oder länger. Die N.C.-Eier zeigen nur einen mässigen Rückgang nach 98 Tagen und manche bleiben bis zu 208 Tagen lebensfähig. Die Ga.-Kultur liegt dazwischen; wenige Eier bleiben nach 139 Tagen Wintertemperatur lebensfähig.

Es wird angenommen, dass Unterschiede in der Widerstandsfähigkeit der *M. javanica*-Kulturen bis zu einem gewissen Grade von den Bedingungen beeinflusst wurden, unter denen die drei Kulturen sich entwickelten.

REFERENCES

Atkinson, G. F. (1889). Nematode root galls. *Bull. Ala agric. Exp. Sta.* **9**, 177-226.

Bessey, E. A. (1911). Rootknot and its control. *Bull. U.S. Bur. Pl. Ind.* **217**, U. S. Government Printing Office, Washington.

Brown, E. B. (1955). Occurrence of the root-knot eelworm. *Meloidogyne hapla,* out of doors in Great Britain. *Nature, Lond.* **175**, 430.

Chitwood, B. G. (1949). Root-knot nematodes. Part 1. A revision of the genus *Meloidogyne* Goeldi, 1887. *Proc. helm. Soc. Wash.* **16**, 90-104.

Colbran, R. C. (1958). Studies of plant and soil nematodes. II. Queensland host records of root-knot nematodes (*Meloidogyne* species). *Qd. J. agric. Sci.* **15**, 101-136.

Cunningham, H. S. (1936). The root-knot nematode (*Heterodera marioni*) in relation to the potato industry on Long Island. *Bull. N. Y. St. agric. Exp. Sta.* **667**, 1-24.

Dropkin, V. H. (1957). Genus *Meloidogyne*-root-knot nematodes. In *"A manual of plant nematology for experiment station workers in the northeastern region"*. Advanced Nematology Worshop. Cornell Univ. July, 1957.

—— (1959). Varietal response of soybeans to *Meloidogyne* — a bioassay system for separating races of root-knot nematodes. *Phytopathology* **49**, 18-23.

Franklin, M. T. (1937). On the survival of *Heterodera marioni* infection out-of-doors in England. *J. Helm.* **15**, 75-76.

Godfrey, G. H. (1934). Indicator plants for measuring soil populations of root-knot nematode, *Heterodera marioni* (Cornu) Goodey. *Soil Sci.* **38**, 3-27.

Godfrey, G. H. & Morita, H. T. (1929). Effects of some environmental factors on the root-knot nematode. *Phytopathology* **19**, 83-84 (Abstr.)

Goplen, B. P., Stanford, E. H. & Allen, M. W. (1959). Demonstration of Physiological races within three root-knot nematode species attacking alfalfa. *Phytopathology* **49**, 653-656.

Hoshino, H. M., & Godfrey, G. H. (1933). Thermal death point of *Heterodera radicicola* in relation to time. *Phytopathology* **23**, 260-270.

Kincaid, R. R. (1946). Soil factors affecting incidence of root-knot. *Soil Sci.* **61**, 101-109.

Linde, W. J. van der (1956). The *Meloidogyne* problem in South Africa. *Nematologica* **1**, 177-183.

Martin, W. J. (1954). Parasitic races of *Meloidogyne incognita* and *M. incognita acrita. Plant. Dis. Reptr Suppl.* **227**, 86-88.

Moore, E. L., Powell, N. T. & Gwynn, G. R. (1961). Root-knot resistance in flue-cured tobacco. *Res. & Fmg N.C.* (in press).

Neal, J. C. (1889). The root-knot disease of the peach, orange, and other plants in Florida, due to the work of *Anguillula. Bull. U.S. Bur. Ent.* **20**, 31 pp.

Powell, W. M. (1957). Variations in host-parasite relationships of the root-knot nematode, *Meloidogyne hapla* Chitwood. Masters thesis. North Carolina State College, N.C.

—— (1960). Efficacy of sampling and assay techniques in estimating plant parasitic nematode populations in field soils. Ph. D. thesis, North Carolina State College, N.C.

Riggs, R. D., & Winstead, N. N. (1959). Studies on resistance in tomato to root-knot nematodes and on the occurrence of pathogenic biotypes. *Phytopathology* **49**, 716-724.

Santmyer, P. H. (1955). A comparison of the thermal death time of two dissimilar species of nematodes: *Panagrellus redivivus* (Linn. 1767) Goodey 1945, and *Meloidogyne incognita* var. *acrita,* Chitwood 1949. *Proc. helm. Soc. Wash.* **22**, 22-25.

Sasser, J. N., & Nusbaum, C. J. (1955). Seasonal fluctuations of host specificity of root-knot nematode populations in two-year tobacco rotation plots. *Phytopathology* **45**, 540-545.

Scholander, P. F., Flagg, W., Walters, V. & Irving, L. (1953). Climatic adaptation in arctic and tropical poikilotherms. *Physiol. Zool.* **26**, 67-92.

Sparck, R. (1936). On the relation between metabolism and temperature in some marine lamellibranches, and its zoogeographical significance. *K. danske vidensk. Selsk.,* **13**, 1-27.

Taylor, A. L., & Buhrer, E. M. (1958). Preliminary report on distribution of root-knot nematode species in the United States. *Phytopathology* **48**, 464 (Abstr.).

Taylor, A. L., Dropkin, V. H., & Martin, G. C. (1955). Perineal patterns of root-knot nematodes. *Phytopathology* **45**, 26-34.

Thomason, I. J. (1957). Influence of soil temperature on reproduction of *Meloidogyne* spp. *Phytopathology* **47**, 34-35 (Abstr.).

Triantaphyllou, A. C., & Sasser, J. N. (1960). Variation in perineal patterns and host specificity of *Meloidogyne incognita. Phytopathology* **50**, 724-735.

Tyler, J. (1933a). Development of the root-knot nematode as affected by temperature. *Hilgardia* **7**, 391-415.

—— (1933b). (Revised 1944). The root-knot nematode. *Circ. Calif. agric. Exp. Sta.* 330.

Walker, J. T. (1960). The effect of hot water at different temperatures on larvae of various species of *Meloidogyne. Phytopathology* **50**, 658 (Abstr.).

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ROOT ROT OF BANANAS CAUSED BY *RADOPHOLUS SIMILIS* (COBB) AND ITS CONTROL IN NEW SOUTH WALES

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The occurrence of *Radopholus similis* on bananas (*Musa cavendishii*) in New South Wales is reported and the symptoms and economic importance of a root rot which the nematode causes are described. The histopathology of root lesions and the role of the fungi *Fusarium oxysporum* and *Rhizoctonia solani* as secondary colonists are examined. Reproduction on *M. acuminata banksii* and *Saccharum officinarum*, but not on *Citrus* spp. showed *R. similis* to be of the "banana" race. Root rot was controlled for 6 months by preplant soil fumigation with the nematicides methyl bromide, D-D, Vapam or Nemagon but re-infestation occurred after 11 months. *R. similis* was not found in virgin soils, but was introduced by planting infested sets. Sets less than 13 cm diameter were disinfested by immersion in water at 55° C for 20 min. without abnormal set losses. Chemical dipping of sets failed to disinfest them. A hot water bath and a method for the bulk treatment of sets are described.

Fruit production and the bearing life of plantations of bananas, *Musa cavendishii* Lambert (varieties Dwarf Cavendish and Williams) in New South Wales (N.S.W.) are reduced by a root rot disease which is widespread and of considerable economic importance.

Healthy banana plants have a shallow but prolific root system (Summerville, 1941) with roots mainly confined to the upper 30 cm of soil but extending to 6 m laterally. In plants affected with root rot small, lenticular sunken lesions develop on young cord (primary adventitious) roots. On older roots, surface cracks appear and when cut longitudinally (Fig. 1) these show as extensive reddish-brown lesions in the cortex. These lesions are followed by decay and death of the root distal to the lesion. The extent of the root system is thus reduced and in severely affected stools 1 year after being established less than 10 % of the roots may have a functional length of more than 60 cm. This results in reduced bunch weights, and the plants do not respond to fertilizers and irrigation. Where root loss is considerable, plants are poorly anchored and under the weight of a maturing bunch of fruit may be uprooted, especially during wet and windy weather (Fig. 2).

Root rot in some banana growing areas of N.S.W. was referred to by Magee (1927), who, in an investigation of bunchy top disease, isolated *Rhizoctonia*

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solani and two species of *Fusarium* from banana root lesions. The banana weevil borer, *Cosmopolites sordidus* Germ., has been suggested as a cause of root rotting. However, the now general use of dieldrin (Braithwaite, 1958) has resulted in the virtual eradication of this weevil but the root knot disease has not decreased in severity and its separate identity has emerged more clearly.

Examination of affected roots has led to the conclusion that the disease root rot, is caused by the burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949 (= *Tylenchus similis* Cobb).

Other nematodes found associated with bananas in N.S.W. are *Meloidogyne javanica* (Treub), *M. incognita* (Kofoid & White), *Helicotylenchus multicinctus* (Cobb), *Xiphinema* sp. and *Trichodorus* sp. No evidence has yet been obtained that these injure the plant.

The origin of the banana plantations in N.S.W. can be traced to sets introduced from Fiji between 1860 and 1910. Cobb (1915) described *Tylenchus similis* from root lesions on bananas and sugar cane (*Saccharum officinarum* L.) from Fiji and it is probable that plants imported to Australia were infested.

Diseases of bananas attributed to *R. similis* have been described by Ashby (1915), Leach (1958), Loos & Loos (1960b), Mallamarie (1939) and of Manilla hemp (*M. textilis* Née) by Van der Vecht (1950) and Taylor & Loegering (1953). *R. similis* probably now occurs in most areas where bananas are grown commercially. Spreading decline of citrus in Florida, U.S.A. and yellows disease of pepper (*Piper nigrum* L.) in Banka, Indonesia (van der Vecht, 1950) are also caused by this nematode. It has not been recorded on *Citrus* spp. or other hosts in Australia.

In the text the term "spearpoint" is used to describe a sucker bearing narrow sword leaves, "bit" a piece of corm with at least one viable bud and a "peeper" a very young sucker bearing scale leaves only. The three types of propagating material have been referred to collectively as "sets".

EXPERIMENTAL

Histopathology of root lesions

Longitudinal and transverse hand sections of young roots showing lesions at various stages of development were stained with lactophenol-cotton blue (Goodey, 1957) and mounted in glycerine. Adult females, larvae and eggs of *R. similis* were found in lesions in the root cortex in the sections examined. Each lesion showed evidence of having originated as a puncture in the epidermis of the root. Beneath the puncture was a zone of necrotic cells containing few nematodes but many eggs, often with prominent embryos. The nematodes occurred mostly in advance of the region of necrosis. In this area cells were enlarged, abnormal tangential division had occurred and the intercellular movement of nematodes and their feeding on cell protoplasm had ruptured cell walls and disorganized the tissue. In a third zone, which formed the boundary to the lesion, mild hypertrophy was evident, but no nematodes were present (Fig. 3).

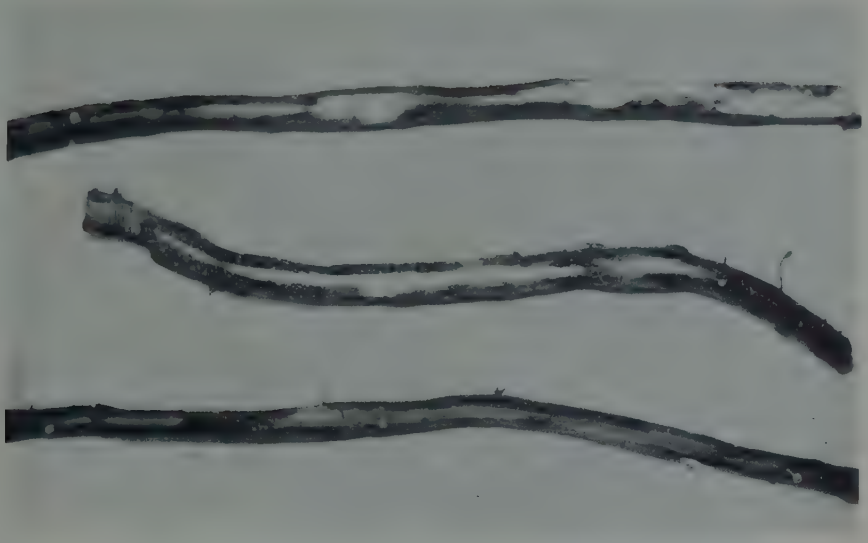

Fig. 1. Cord roots of banana plant, cut longitudinally, showing lesions in the cortex and some secondary invasion of the stele.

Fig. 2. Part of plantation of Cavendish bananas infested with *R. similis*, showing bunched plants uprooted.

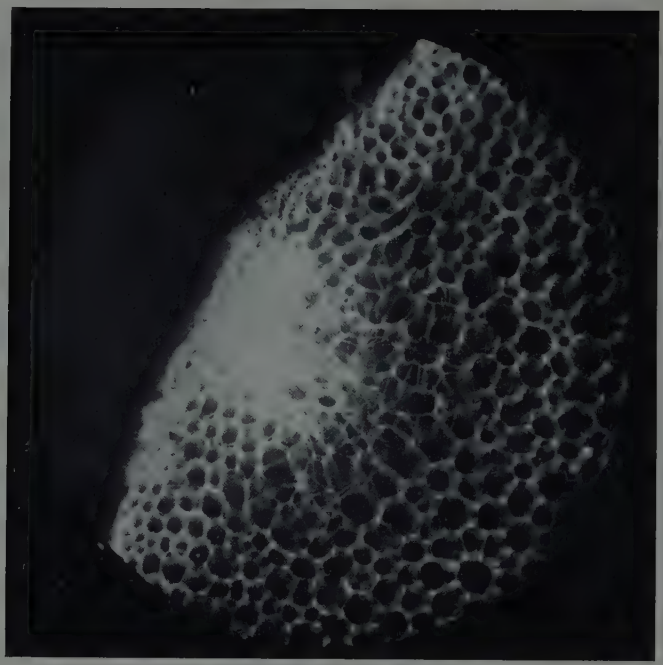

Fig. 3. Transverse section through lesion in cord root, showing necrosis and histogenesis.

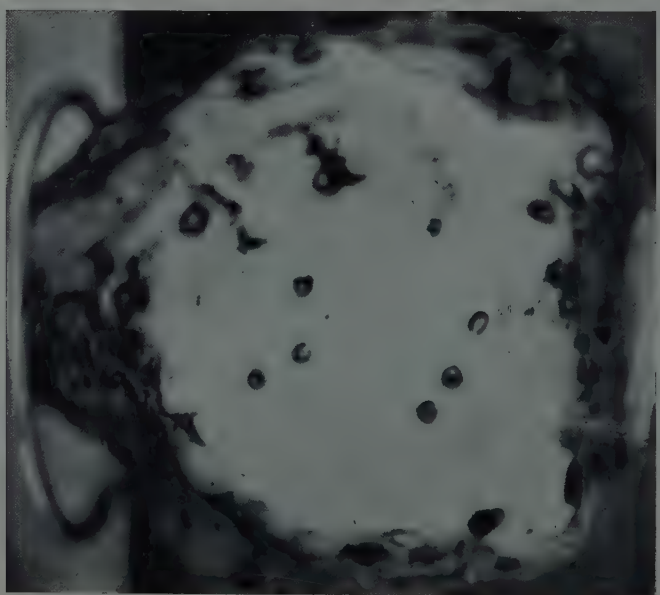

Fig. 4. Spearpoint cut to expose embedded cord roots infested with *R. similis*.

The lesions resemble those described by DuCharme (1959) on citrus roots. He described hypertrophy and tangential division of cells, with no necrosis, in the pericycle of grapefruit roots invaded by *R. similis* under aseptic conditions.

The lesions were extended in the cortex of banana roots by tunnelling of nematodes which probably do not move out of a root so long as they are able to invade healthy tissue. The stele was not invaded by the nematodes, the thickened endodermis possibly acting as a barrier. Where necrosis had advanced to the endodermis, sections stained with lactophenol-cotton blue showed fungal hyphae in the stele.

Cord roots arise endogenously and, in a large corm, may pass through 7 to 10 cm of cortex before emerging. Diffuse lesions in the cortex of corms similar to those described by Loos & Loos (1960b) surround these emerging roots and surface wounds (Fig. 4).

Eleven genera of fungi were isolated by plating dissected tissue from the necrotic zone of root lesions on to potato dextrose agar, but only *R. solani* and a form of *F. oxysporum* were consistently obtained.

The pathogenicity of these fungi to seedlings of *Musa acuminata* Colla subsp. *banksii* was tested in the presence and absence of *R. similis* in a pot experiment

TABLE I

Development of root rot in seedlings of Musa acuminata banksii *following inoculation with* Fusarium oxysporum, Rhizoctonia solani *and* Radopholus similis

Inoculum	Number of Rotted Roots / Total Number of Roots per Plant					Mean % Healthy Roots/ Plant
	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	
Control (Uninoculated)	0/16	0/18	0/12	1/14	1/17	97
Fusarium oxysporum *(2%)	0/12	1/15	0/16	0/18	2/12	96
Rhizoctonia solani *(2%)	1/23	0/12	0/18	1/11	2/14	95
Radopholus similis (4,000 per pot)	12/16	8/18	6/12	5/17	7/15	51
Radopholus similis (4,000 per pot) + *Fusarium oxysporum* *	8/22	10/16	2/12	4/14	6/18	63.5
Radopholus similis (4,000 per pot) + *Rhizoctonia solani* *	9/17	10/22	10/16	6/16	12/19	52.2

* 2 g maize meal sand inoculum/100 g sand.

in a greenhouse. Maize meal sand cultures of six isolates of each of the two fungi were prepared and used to inoculate pots of sterilized soil. Inoculum of *R. similis* was obtained from banana roots by incubation in water (Young, 1954). The nematodes were washed twice in distilled water and added to the soil of pots to be tested. Four week old seedlings raised in sterile sand were planted into the inoculated pots, watered weekly with a nutrient solution and, after 8 weeks, the numbers of healthy and diseased roots per plant were counted.

The combinations of inoculum used and the results obtained are given in Table I.

Identification of the race of R. similis *in N.S.W.*

DuCharme & Birchfield (1956) suggested the existence of three physiological races of *R. similis* morphologically similar, but differing in their ability of infect the roots of citrus and bananas. Further evidence for the existence of physiologic races is provided by the work of Van Weerdt (1957), who failed to obtain infection of citrus with *R. similis* from bananas.

Citrus trees, principally rough lemon and Rangpur lime occur as volunteer seedlings in banana plantations in N.S.W.

The roots of such trees from plantations known to be infested with *R. similis* have been examined. No infection of citrus roots by *R. similis* was detected.

Experimental inoculation of citrus seedlings under controlled conditions was attempted. Cobb (1915) recovered *R. similis* from lesions on sugar cane in Fiji and as bananas may be planted in N.S.W. into land which has grown sugar cane, this plant has been included in the present study. Eight pots each of banana raised from seed, sugar cane from stem cuttings and the following citrus seedlings were established in sterilized sandy loam and grown in a greenhouse: *Citrus aurantium* L. (Sour orange, Var. Rough & Bitter-sweet), *C. limon* (L.) Burm. f. (lemon, Var. Lisbon, Rough and Meyer), *C. reticulata* Blanco (Mandarin, Var. Cleopatra, Wallent, Thorny, Emperor, Ellendale), *C. grandis* (L.) Osbeck (Shaddock), *C. paradisi* Macfad. (Grapefruit, var. Marsh, Leonardy), *Poncirus trifoliata* (L.) Raf., *C. medica* L. (Citron, Var. Knight) and *C. aurantifolia* Swingle (Rangpur lime). *R. similis* from roots of *M. cavendishii* were collected in four banana growing areas of N.S.W.

Two pots of each test plant were inoculated with nematodes from each area at the rate of 4,000 nematodes per pot. Four months later the roots of each plant were examined for lesions.

No root lesions were found on any of the citrus species, but numerous lesions had developed on the roots of banana and sugar cane.

To compare the rate of multiplication of *R. similis* on citrus, banana and sugar cane, five test plants were selected for this experiment and eight pots of each were established. Each pot was inoculated with 4,000 nematodes taken from banana roots and at intervals of 6 weeks two plants were removed and the number of nematodes in 25 g root and 100 g soil samples, determined. The results are given in Table II.

TABLE II

Numbers of Radopholus similis *recovered from roots of* Citrus *spp.*, Musa acuminata banksii *and* Saccharum officinarum *at intervals after inoculation*

Test Plant	Number of *R. similis* extracted							
	6 weeks		12 weeks		18 weeks		24 weeks	
	Root	Soil	Root	Soil	Root	Soil	Root	Soil
Citrus medica (Citron, Var. Knight)	24	104	4	82	0	16	0	0
C. limon (Lemon, Var. Rough)	40	180	12	74	0	56	0	0
C. paradisi (Grapefruit, Var. Marsh)	64	240	26	84	12	10	0	0
Musa acuminata banksii (Banana)	32	210	92	180	140	260	320	580
Saccharum officinarum (Sugar cane)	18	190	69	210	86	300	104	360

Although *R. similis* from roots of bananas invaded the roots of *Citrus* spp., banana and sugar cane plants in similar numbers, the nematode did not reproduce on citrus and the population in pots declined until, after 24 weeks, none was recovered from root or soil samples. In contrast, the numbers of nematodes obtained from sugar cane and bananas increased in the period of the experiment. Birchfield (1957) found that *R. similis* from citrus did not survive for more than 4 months in soil in the absence of a host.

As *R. similis* has not been reported on citrus in Australia and as it has not been possible to inoculate citrus seedlings with inoculum from bananas, it is probable that the nematode which occurs on bananas belongs to the "banana" race of DuCharme and Birchfield.

Method of field spread

Although there is no evidence that *R. similis* occurs in soils in N.S.W. or Queensland in which *Musa* spp. or sugar cane have not been grown, plantations established in virgin soils may show root rot within 3 months of establishment. Examination of planting material has shown that infestation with *R. similis* is common. It is probable therefore that spread of the disease occurs by this means.

An experiment was carried out to show that either infested soil or sets can be the source of infestation of a plantation. Infested spearpoints each weighing less than 3 kg were divided into three groups, one remained untreated, one group was thoroughly pared of diseased outer leaf bases after the method of Loos & Loos (1960a) and one group was subjected to a hot water treatment of 20 min at 55° C. The sets were planted singly in pots of infested soil, virgin soil and soil sterilized by methyl bromide, D-D or steam. Three pots were used for each treatment. After

3 months root samples were taken, bulked for each soil-set treatment and macerated in a homogenizer for 3 min. Nematodes were recovered from the debris by sieving and the results are shown in Table III.

TABLE III

The effect of soil and set treatments on the incidence of root rot

Soil Treatment	Set Treatment		
	Immersion in water at 55° C for 20 min.	Thorough paring	No treatment
Untreated soil from infested plantation	+	+	+
Untreated soil from infested plantations destroyed 3 months previously	+	+	+
Untreated, virgin soil	—	—	+
Soil from infested area treated with methyl bromide (1 kg/100 sq. m. for 24 hr)	—	—	+
Soil from infested area steam sterilised	—	—	+
Soil from infested area treated with D-D (455l/ha)	—	—	+

\+ *R. similis* present
— *R. similis* absent

The results showed that infestation developed in roots produced by infested sets regardless of the condition of the soil in which they were planted. No infection developed in roots produced by disinfested sets planted in virgin soil or in sterilized soil.

It is clear that the use of infested sets could be the means by which *R. similis* is introduced into plantations on virgin soil and the reason for its wide distribution. Dispersal could be aided by the common practice in N.S.W. of taking sets, not from the vigorous areas in plantations, but from those which are unproductive, since the usual reason for unproductiveness is nematode infestation.

Fumigation of infested soil

An area of 2.5 ha where infested bananas had been destroyed by a hormone herbicide (Jeater, Cann & Eastwood, 1951) 3 months previously, was used to test the efficiency of nematicides. Persisting corms were removed, the area cultivated and laid out into 25 plots (5 × 5 treatments), each 6 m × 6 m (20 ft × 20 ft) and a buffer zone 3 m (10 ft) wide treated with D-D at 455 l/ha (40 gal/acre).

Five treatments, methyl bromide (rate 1 kg/10 sq m = 2 lb/100 sq ft), D-D (455 l/ha = 40 gal/acre), Vapam (1138 l/ha = 100 gal/acre), Nemagon (57 l/ha = 5 gal/acre) and no treatment were arranged in a randomized blocks design. Vapam, D-D and Nemagon were injected at intervals of 30 cm and methyl

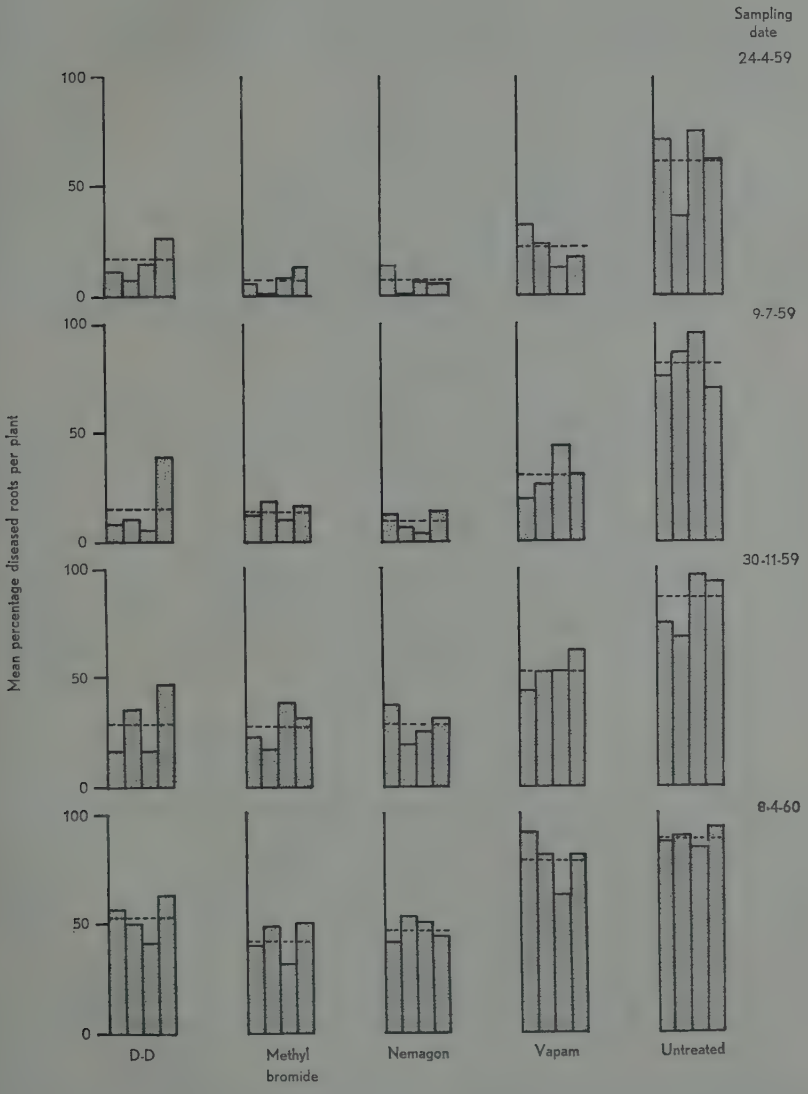

Fig. 5. The incidence of root rot after treatment with four soil fumigants estimated at four sampling dates. The mean of four replicates is shown by a broken line.

bromide was released as a gas under plastic sheeting. The plots were rolled after treatment but the steep slope and stony surface prevented effective sealing.

Three weeks after treatment thirteen spearpoints (Var. Dwarf Cavendish) treated in water at 55° C for 20 min were planted in each plot. At intervals of approximately 3 months three plants were removed from each plot and the percentage of diseased roots in each plot assessed. The results are set out in Fig. 5.

Samplings during the first 6 months showed that considerable control of root rot had been attained in all treated plots, but after 11 and 16 months, root rot in all plots was severe.

The disinfestation of planting material

Loos & Loos (1960a) showed that nematode-free sets could be obtained by paring all discoloured outer tissue away and dipping in a Bordeaux mixture-Nemagon preparation. Such treatment is tedious and time-consuming though suitable for establishing nurseries from which disease-free sets can later be obtained.

Chemical treatment

Twenty infested spearpoints, each with a maximum diameter less than 13 cm were immersed for 3 hr in one of the following dips: Parathion (0.2 % v/v), Systox (0.05 %), Phosdrin (0.2 %), Ekatin (0.2 %), Meta-iso-Systox (0.2 %), Nemagon (0.2 %), EDB (0.2 %) and Aretan (0.6 % w/v). The sets were stored in a dry place after dipping and 14 days later corm lesions and roots embedded in the corm were sampled and nematode extractions made.

Active *R. similis* were obtained from all samples. Nemagon and E.D.B. at least, are known to kill *R. similis* and failure of all dips to disinfest in this experiment is attributed to inadequate penetration of sets.

Hot water treatment

Treatment in hot water has been for many years a standard method for eliminating of nematodes from propagating material (Staniland, 1933, 1950, 1953).

To arrive at a basis for the experimental treatment of infested banana sets, the temperature required to kill *R. similis in vitro* and the time required for heat to penetrate banana corm tissue were determined. Mallamarie (1939) suggested immersing banana sets infested with *R. similis* in water at 65° C for 5 min, but gave no details of the efficiency of the treatment.

The method described by Staniland (1950), including his modifications for use at temperatures near the death point of the nematodes was used to determine the temperature lethal to *R. similis* in water. Approximately 200 nematodes were used for each exposure. After heating these were placed in watch glasses, stored at 10° C and examined over 5 days for activity. The results are shown in Fig. 6.

All nematodes were killed by exposure to 50° C for 90 sec. Birchfield (1954),

however, found that a hot water treatment of 6 min at 50° C was necessary to kill *R. similis* from citrus. This difference in time required for killing may be due to the different techniques used, or it is possible that differences in heat tolerance may occur between strains. Christie & Crossman (1935) found that "strains" of *Aphelenchoides fragariae* differed in their heat tolerance.

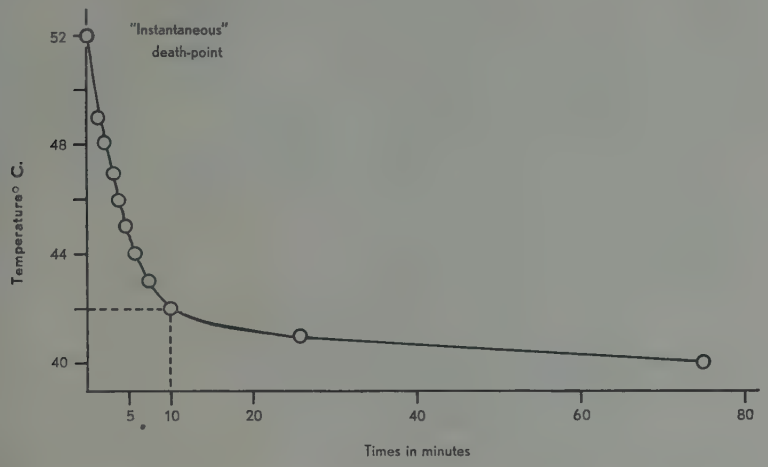

Fig. 6. Curve showing the time at different temperatures required to kill *Radopholus similis* in water.

The rate of penetration of heat through banana corm tissue during hot water treatment was determined by inserting a thermometer into the tissue of spearpoints and bits so that the bulb was midway between the surface of the corm and the outer limit of the corm cortex. In previous samplings of infested corms, nematodes were not found deeper on the corm than this region. The sets each with a thermometer were immersed singly in a constant temperature water bath, capacity 109 l (24 gal.) and the temperature of each set was recorded at 10 min. intervals over 2 hr.

The same sets were used at five bath temperatures, the sets being air-dried at 12° C for 24 hr between each series of recordings. The heat gained by a spearpoint and a bit is shown in Fig. 7.

The effect of the size of planting material on rate of heat penetration was similarly found by observations on material of several sizes (Fig. 8). The effect of set size on heat penetration is considerable. In a bath at 55° C, a temperature of 42° C was recorded after approximately 16 min for spearpoints 12.7 cm diameter or less and in quartered corms. In larger spearpoints and in halved and whole corms, 42° C was not reached in 30 min.

To study the effect of hot water treatment on nematode survival and set viability in large samples of planting material, a tank was constructed with sufficient capacity to provide treated sets for commercial scale planting. The tank was similar in con-

struction to that used by Hughes (1954) for the treatment of sugar cane sets. It was of insulated steel (Fig. 9) capacity 4,550 l (1,000 gal.), the water was heated by low pressure steam and circulated by a 6.4 cm (2½ in.) centrifugal pump. The

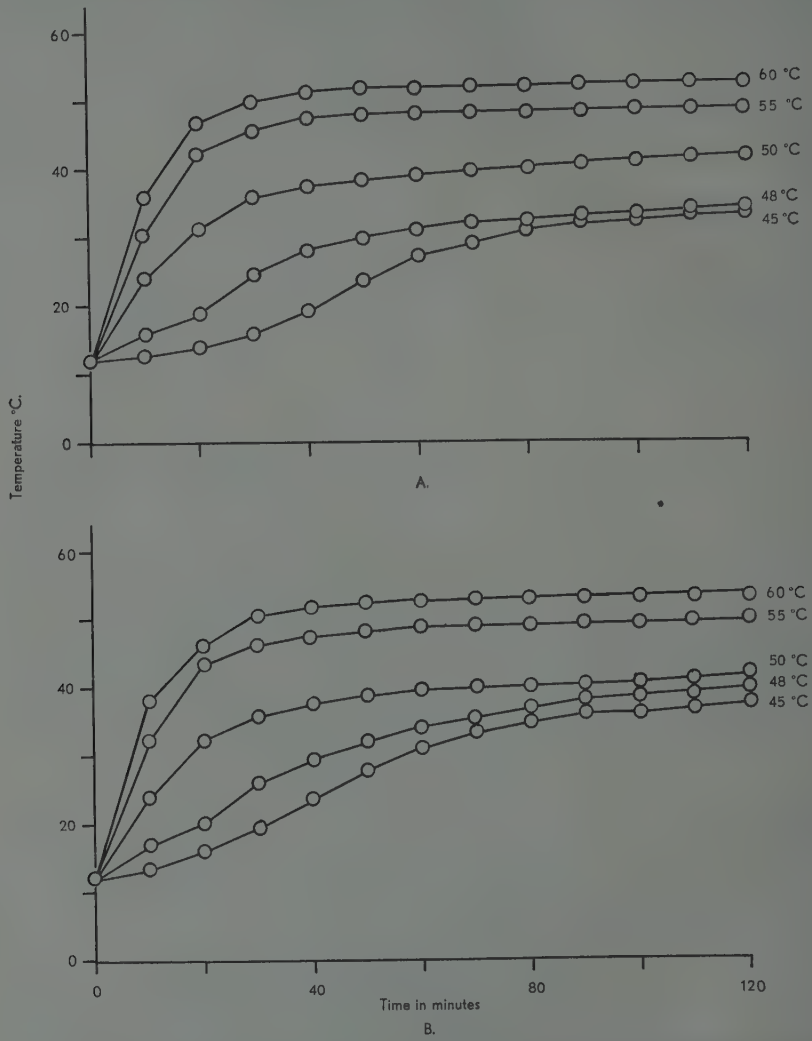

Fig. 7. Curves showing the increase in temperature within the same (A) spearpoint and (B) bit, when immersed in a water bath at five temperatures.

temperature of the water in the tank was measured on a thermometer with a long mercury column immersed to 1 m. The quantity of steam entering the tank was thermostatically controlled.

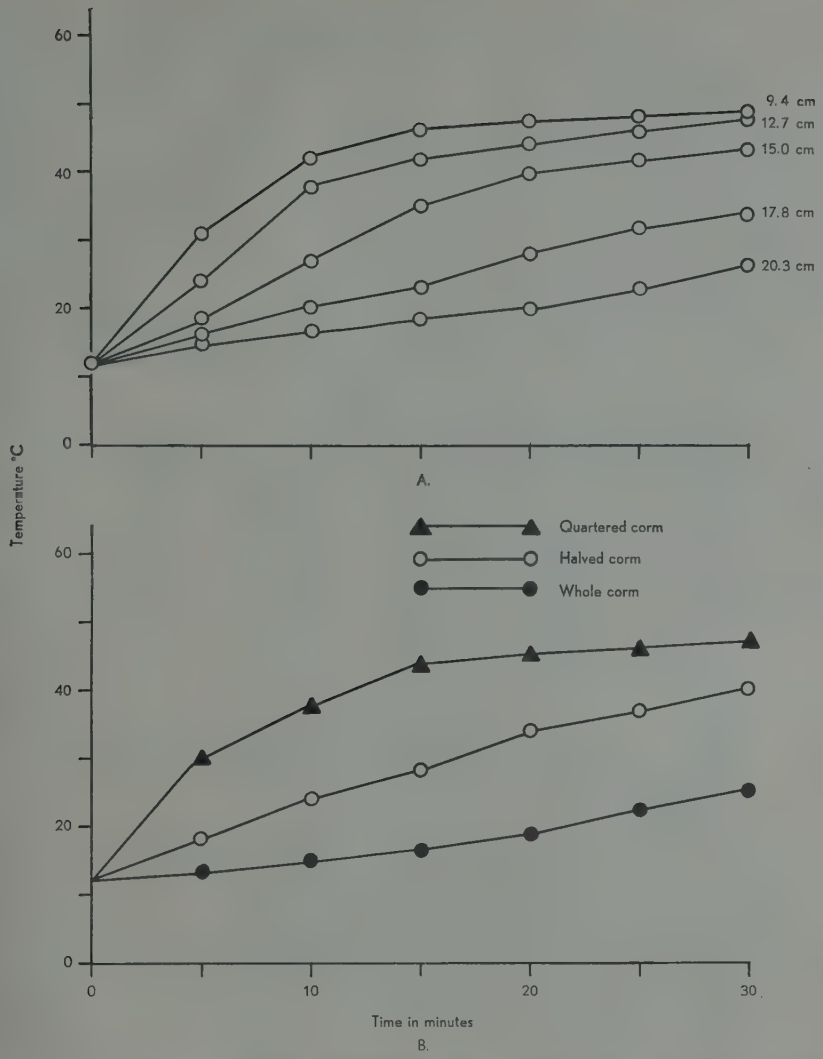

Fig. 8. Curves showing the increase in temperature within (A) spearpoints of different diameters and (B) whole, halved and quartered corms, when immersed in a water bath at five temperatures.

During treatment sets were held in wire baskets 45 × 45 × 52 cm, made of 1.3 cm galvanised water piping covered with chain wire of mesh 2.5 cm and fitted with a hinged mesh lid to prevent sets floating out when submerged. Baskets holding 40 to 70 sets were lifted singly into the tank by a small electric hoist. The baskets rested on a steel grid, 22 cm from the tank floor. When fully loaded the

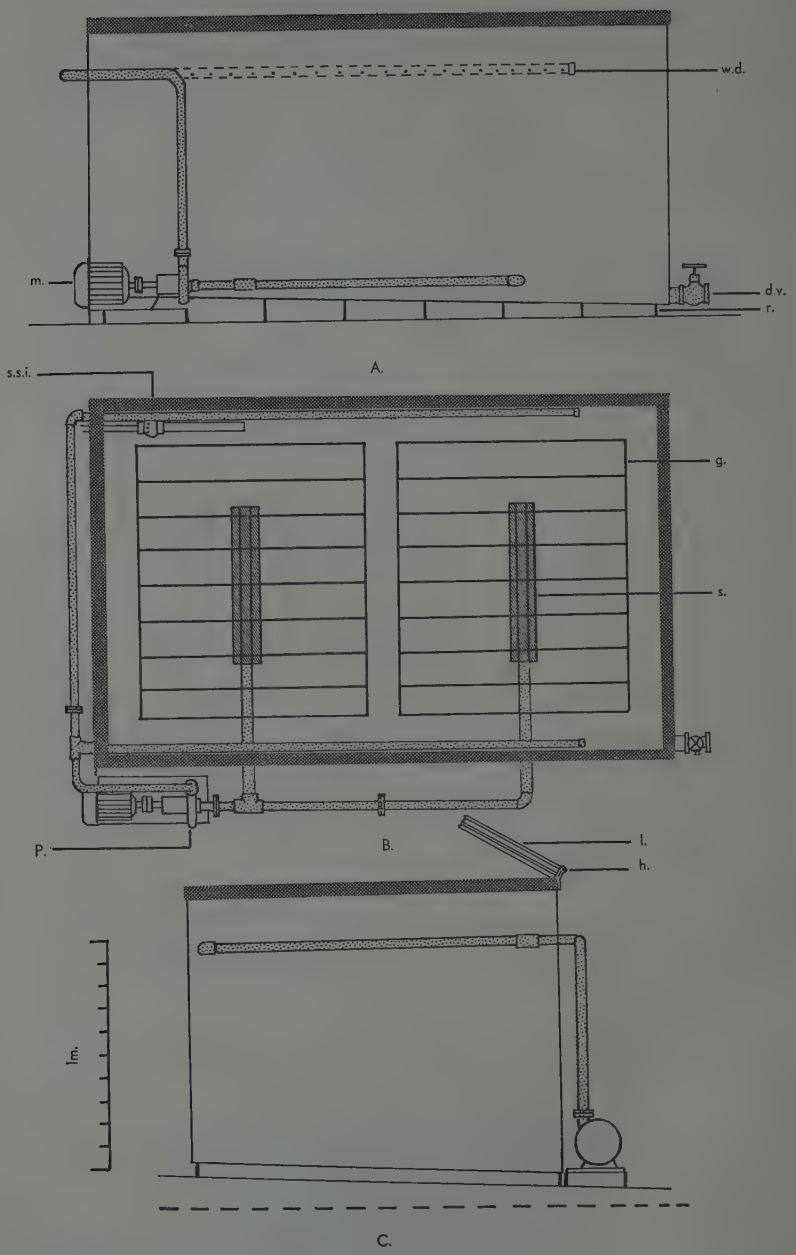

Fig. 9. Legend see next page.

tank held eight baskets separated from each other by approximately 13 cm of water. During treatment the tank was closed with three wooden lids.

The effects of hot water treatment on set and nematode survival were examined in infested planting material of three types (spearpoints, peepers and bits) for six combinations of temperature and immersion time. The sets were all less than 13 cm in diameter and washed free of adhering soil before treatment. Injury was assessed by examining terminal or axillary buds 14 days after treatment. Nematode survival was assessed by examination of samples of root and corm lesions after 14 days.

Treatments and results are shown in Table IV.

TABLE IV

The effect of heat treatment of infested banana sets on survival of sets and Radopholus similis

Hot water Treatment Temperature & Time	Survival of Sets			Survival of *R. similis* **		
	Spearpoints	Peepers	Bits	Spear-points	Peepers	Bits
45° C 120 mins.	*122/215	28/53	96/232	+	+	+
48° C 90 mins.	114/125	14/15	140/155	—	—	+
55° C 20 mins.	187/192	32/34	116/120	—	—	—
55° C 30 mins.	70/94	10/30	65/100	—	—	—
60° C 10 mins.	122/130	15/16	94/100	+	—	+
60° C 20 mins.	4/50	4/16	11/96	—	—	—

* No. sets surviving after 14 days / Total No. treated.
** + Active *R. similis* recovered after 14 days.
— No *R. similis* recovered after 14 days.

DISCUSSION

Plantations infested with *R. similis* in N.S.W. usually persist for about 4 years before their continued cultivation becomes unprofitable. Areas replanted with bananas within 1 year of removing the preceding crop, are slow to establish and may remain unthrifty throughout their growth. In Western Australia where bananas are grown on alluvial soils at Carnarvon, infested plantations may degenerate so rapidly that first ratoon bunches are not harvested (J. A. Lawson, *in litt.*).

Fig. 9. Tank for bulk treatment of banana sets; (a) side, (b) internal and (C) end views.

w.d. water discharge pipe on inside of tank
d.v. 6.3 cm drainage valve
m. 2 h.p. electric motor
s.s.i. silent steam injector
g. steel grid 23 cm from bottom of tank
s. perforated suction pipe covered with wire mesh
p. 3.8 cm centrifugal pump
l. wooden lid
h. steel hinge

In N.S.W., leaf fall (Magee & Fitzpatrick, 1932) can devastate first crops of bananas grown on land previously cropped with sugar cane or grass pasture. The disease at first resembles root rot, but later, sudden defoliation occurs with the onset of winter conditions or as the bunch matures. The cause of this disease is unknown, but the symptoms and etiology suggest that it may be a severe reaction to *R. similis* infestation.

In the pathogenicity tests described neither *F. oxysporum* nor *R. solani* caused lesions, but lesions and root destruction occurred in all pots into which *R. similis* was introduced. The number of roots with lesions when *F. oxysporum* or *R. solani* and *R. similis* were used together was no greater than when *R. similis* was used alone. Both fungi did not cause lesions in the absence of *R. similis*. These results indicate that *R. similis* is the primary cause of banana root rot in N.S.W. It is probable that the root wounds caused by its invasion would be quickly colonised by soil inhabiting fungi, such as *Rhizoctonia* and *Fusarium* which have a high competitive saprophytic ability. Mountain (1954), working with *Pratylenchus* spp. on tobacco under aseptic conditions, considered invasion by saprophytes essential for typical lesions to develop. When either *Fusarium* or *Rhizoctonia* were present, lesions caused by *R. similis* on banana roots enlarged more rapidly. Invasion of the stele of the root by fungi occurred, probably causing a more rapid atrophy of the root.

The observation that similar numbers of the banana race of *R. similis* invaded the roots of banana, sugar cane and citrus plants but did not multiply in citrus plants agrees with to the observations made for races of *Ditylenchus dipsaci* (Kühn) on test plants used to distinguish races and for a given race of *D. dipsaci* on susceptible and resistant varieties. Field surveys carried out in Australia have not detected plants other than sugar cane in *Musa* spp. as hosts of *R. similis*. This suggests that the banana race may have few hosts compared with the citrus race which has been shown to have a wide host range.

The development of root rot in fumigated soils indicated that fumigation had been inefficient. A preplanting fumigation alone and the use of nematode-free planting material would therefore appear to be inadequate for the establishment of disease-free plantations in infested soil. Post-planting fumigation, possibly annually, would be required in addition and the cost would probably make this uneconomic. In the N.S.W. the land usually used for banana plantations is steep and the surface of the soil stony and often strewn with logs or divided by watercourses. This makes thorough application difficult and the use of machinery for applying nematicides often impossible.

The apparent absence of *R. similis* from virgin areas or areas freed of *Musa* spp. or sugar cane for more than 2 years has been noted. If such areas could be planted with clean sets, degeneration as a result of root rot should be delayed or even prevented. As this method of control would be cheap and would not interrupt plantation routine, an attempt has been made to develop a practical technique for the disinfestation of sets. As a result of these experiments a treat-

ment time and temperature of 55° C for 20 min was selected as giving effective control with the minimum of loss, and the method was considered worthy of trial on a field scale.

In 1958 and 1959 small areas planted with heat-treated sets established more quickly than those planted with untreated material but no yield comparisons are available. In 1960 a hot water tank similar to that shown in Fig. 5 was constructed and put into operation at Murwillumbah in the Tweed River area, N.S.W. A total of 45,000 sets were treated in the November-December planting period. Only sets to be planted in virgin areas or areas which had not been growing bananas or sugar cane for more than 10 years were accepted for treatment. No results as yet are available.

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RÉSUMÉ

Sur la pourriture des racines du bananier causée par Radopholus similis *(Cobb) et la lutte contre cette maladie en Australie*

L'auteur signale la présence de *Radopholus similis* dans des bananiers (*Musa* spp.) de New South Wales et décrit les symptômes ainsi que l'importance économique pour cette région d'une pourriture des racines causée par ce nématode sur *M. cavendishii*. Il présente une étude histopathologique des lésions des racines et examine le rôle des champignons *Fusarium oxysporum* et *Rhizoctonia solani* comme parasites secondaires. La reproduction de *R. similis* étant obtenue sur *M. acuminata banksii* et sur *Saccharum offinarum* mais non sur *Citrus* montre que ce nématode appartient à la „race des bananes". La pourriture a pu être combattue pendant six mois par fumigation du sol avant la plantation à l'aide des nématicides (bromure de méthyle, D-D, Vapam, ou Nemagon), mais avec tous ces produits une ré-infestation a lieu après onze mois. *R. similis* n'a pas été identifié dans les sols vierges, mais fut introduit en plantant des rejets contaminés. Des rejets d'un diamètre inferieur à 13 cm furent désinfectés par immersion dans l'eau à 55° C pendant 20 minutes sans pertes anormales de rejets. L'immersion chimique des rejets n'a donné aucun résultat.

L'auteur donne une description d'un appareil (bain marie) et d'une méthode pour le traitement de grandes quantités de rejets.

REFERENCES

Ashby, S. F. (1915). Notes on cultivated crops observed in 1913/14 — Banana Diseases. *Bull. Dep. Agric. Jamaica* **2**, 316-317.

Birchfield, W. (1954). The hot water treatment of infested nursery stock. *Proc. Fla. hort. Soc.* **67**, 94-96.

—— (1957). Observations on the longevity without food of the burrowing nematode. *Phytopathology* **47**, 161-162.

Braithwaite, B. M. (1958). Ground spraying for the control of banana weevil borer. *J. Aust. Inst. agric. Sci.* **24**, 27-34.

CHRISTIE, J. R. & CROSSMAN, L. (1935). Water temperatures lethal to begonia, chrysanthemum and strawberry "strains" of the nematode, *Aphelenchoides fragariae*. *Proc. helm. Soc. Wash.* **2**, 98-103.

COBB, N. A. (1915). *Tylenchus similis*, the cause of a root disease of sugar cane and banana. *J. agric. Res.* **4**, 561-568.

DUCHARME, E. P. (1959). Morphogenesis and Histopathology of lesions induced on Citrus roots by *Radophilus similis*. *Phytopathology* **49**, 388-395.

DUCHARME, E. P. & BIRCHFIELD, W. (1956). Physiological races of the burrowing nematode. *Phytopathology* **46**, 615-616.

GOODEY, J. B. (1957). *Laboratory methods for work with plant and soil nematodes*. *Techn. Bull. Min. Agric.* No. 2, 3rd ed. London H.M.S.O., 47 pp.

HUGHES, C. G. (1954). The control of ratoon stunting disease. *Int. Sug. J.* **56**, 338-340.

JEATER, J. G., CANN, H. J. & EASTWOOD, H. W. (1951). Hormones used to destroy bananas. *Agric. Gaz. N.S.W.* **62**, 140-144.

LEACH, R. (1958). Blackhead Toppling disease of bananas. *Nature Lond.* **181**, 204-205.

LOOS, C. A. & LOOS, SARAH, B. (1960a). Preparing nematode free banana seed. *Phytopathology* **50**, 383-386.

—— & —— (1960b). The blackhead disease of bananas. *Proc. helm. Soc. Wash.* **27**, 189-193.

MAGEE, C. J. P. (1927). Investigations on the bunchy top disease of the banana. *Bull. Coun. sci. indust. Res.* **30**.

MAGEE, C. J. P. & FITZPATRICK, A. C. (1932). Leaf fall of bananas. *Agric. Gaz. N.S.W.* **43**, 319-321.

MALLAMARIE, A. (1939). La pourriture vermicularie du bananier de Chine. *Agron. colon.* **254**, 33-75.

MOUNTAIN, W. B. (1954). Studies of nematodes in relation to brown root rot of tobacco in Ontario. *Canad. J. Bot.* **32**, 737-759.

STANILAND, L. A. (1933). The treatment of Narcissus bulbs with hot water. *J. Minist. Agric.* **40**, 343-355.

—— (1950). Experiments on the control of chrysanthemum eelworm, *Aphelenchoides ritzemabosi*. *Ann. appl. Biol.* **37**, 11-18.

—— (1953). Hot water treatment of strawberry runners. *Plant Path.* **2**, 44-48.

SUMMERVILLE, W. A. T. (1939). Root distribution of the banana. *Qd. agric. J.* 376-392.

TAYLOR, A. L. & LOEGERING, W. Q. (1953). Nematodes associated with root lesions in Abaca. *Turrialba* **3**, 8-13.

VAN DER VECHT, J. (1950). Op planten parasiterende aaltjes. In: L. G. E. Kalshoven: *De Plagen van de Cultuurgewassen in Indonesië*. W. van Hoeve, 's-Gravenhage, p. 16-41.

VAN WEERDT, W. (1957). Studies on the Biology of *R. similis*, Part 1. *Plant Dis. Reptr* **41**, 382-385.

YOUNG, T. W. (1954). An incubation method of collecting migratory endoparasitic nematodes. *Plant Dis. Reptr* **38**, 794-795.

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EFFECT OF PARASITIC NEMATODES ON THE SEVERITY OF COMMON ROOT ROT OF CANNING PEAS [1])

BY

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The severity of root rot of peas caused by the fungus *Aphanomyces euteiches* was increased by the nematode *Tylenchorhynchus martini* when the population was as low as 375 nematodes per 5 inch pot. The increase in root rot following addition of nematodes was present only when a strongly pathogenic isolate of *A. euteiches* was used and this increase was directly related to the number of nematodes added. This effect was not obtained in the presence of a weakly pathogenic isolate of the fungus. The number of parasitic nematodes recovered from the soil was directly related to the number of nematodes added. However, in fallow soil, without peas, the number of *T. martini* recovered after the same period of time was considerably less than in the soil inoculated with the same number of nematodes in the presence of pea plants.

In 1959, several parasitic nematodes were reported to be associated with canning peas and results of preliminary studies indicated that nematodes may be related to the root rot complex of canning peas in Minnesota (Haglund & King, 1959; Wyllie, 1960). Labruyère, Den Ouden & Seinhorst reported that early yellowing of peas was related to the nematodes present in the soil. They determined that neither the nematode *Hoplolaimus uniformus* Thorne nor the fungus *Fusarium oxysporum* f. *pisi* (Linford) race 3 Snyder & Hanson could cause the disease singly, but if both were present, the disease would occur. The disease was controlled either by removing nematodes from the soil mechanically or by treatment of the soil with a selective nematocide.

A survey in Minnesota indicated that nematodes in the genus *Tylenchorhynchus* were very common in the soil of pea-growing area. Therefore, studies were made to determine the relationship of nematodes to the severity of *Aphanomyces* root rot of canning peas.

MATERIALS AND METHODS

One hundred specimens of *Tylenchorhynchus martini* Fielding, isolated from a pea field in Minnesota, were hand picked from the mass population and placed on the roots of corn plants growing in steamed soil. The nematodes were allowed to develop on corn for 6 months and again 100 nematodes were hand picked and placed on the roots of a second group of corn plants. Eighteen months later the second culture of nematodes was examined and found to have approximately 75 percent *T. martini* and 25 percent non-stylet species of nematodes, mostly

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Rhabditis sp. This mixture of *T. martini* and the non-stylet form was added to the soil at concentrations of 500, 1500, and 3000 per 5-inch pot.

Cultures from single zoospores of *Aphanomyces euteiches* Drechs. isolated from pea fields in Minnesota were added to the soil at a concentration of 25,000 zoospores per 5-inch pot. This concentration was below that needed to cause severe root rot in the absence of nematodes. Two isolates of *A. euteiches,* called 2 and 4, were used. Previous studies have shown that isolate 2 was very pathogenic and isolate 4 mildly pathogenic to peas.

Pea seedlings of Perfected Wales were germinated in vermiculite, and after 10 days, five seedlings were transplanted into clay pots of steamed soil free from nematodes. One day after transplanting, the seedlings were inoculated with the nematodes and the isolates of *A. euteiches,* singly and/or in combination at various concentrations. The nematodes were applied to the surface of the soil in 50 ml of 0.1 percent water agar and the zoospores of *A. euteiches* added in 10 ml of water. All of the pots of seedlings received water agar and water, plus another 50 ml of tap water. The plants were grown in a greenhouse maintained at 24° C during the day and 18° at night.

At canning maturity of the peas, the severity of root rot was determined by the method described by Johnson (1953) and the population of nematodes measured by processing 15 g of soil from each of the pots by the centrifuge method described by Caveness & Jensen (1955).

RESULTS

Nematodes in the absence of *A. euteiches* did not cause detectable damage to the peas. In the soils inoculated with isolate 2 of *Aphanomyces* and nematodes,

TABLE I

Effect of Tylenchorhynchus martini *on the severity of root rot of Perfected Wales peas caused by* Aphanomyces euteiches.

Treatment	Root Rot Index [1]	
	Isolate 2 [2]	Isolate 4 [3]
Control	1.1 (± .08)	1.0
Aphanomyces	1.5 (± .29)	1.0
Aphanomyces + 500 nema	2.8 (± .51)	1.0
Aphanomyces + 1500 nema	3.2 (± .40)	1.0
Aphanomyces + 3000 nema	3.5 (± .73)	1.0
500 nema	1.0 (± .04)	1.0
1500 nema	1.0 (± .00)	1.0
3000 nema	1.1 (± .04)	1.0
Nema supernate [4]	1.1 (± .02)	1.0
Fallow [5]	——	—

1) Root rot index determined at canning maturity; isolate 2 is based on 10 replications; isolate 4 based on 5 replications. 1 = no root rot. 4 = severe root rot.

2) LSD (.05) = 1.15, (.01) = 1.68.

3) Standard error of the mean.

4) 50 ml of supernate from the nematode culture applied to each replication.

5) Soil inoculated with 3000 nematodes and not planted.

the increase in root rot was related to the concentration of nematodes added to the soil (Table I). The average root rot index for the soil inoculated with isolate 2 was 1.5. However, with the addition of 500, 1500, and 3000 nematodes the root rot index was 2.7, 3.2, and 3.6, respectively. In soil inoculated with isolate 4 of *Aphanomyces,* no root rot developed in the treatments with or without nematodes.

The number of parasitic nematodes recovered from the soil after the peas were harvested (Table II), was directly dependent upon the number of nematodes

TABLE II

Treatment	Number of *T. martini* per pot [1] Isolate 2	Isolate 4
Population of Tylenchorhynchus martini *in the soil at maturity of Perfected Wales peas.*		
Control	0	0
Aphanomyces	0	0
Aphanomyces + 500 nema	250	300
Aphanomyces + 1500 nema	1000	1000
Aphanomyces + 3000 nema	2550	2000
500 nema	200	300
1500 nema	1200	700
3000 nema	2500	1600
supernate [2]	0	0
fallow [3]	850	600

1) Number of nematodes recovered is based on the results of processing 1/100 of the sample by centrifuging. Average, for isolate 2 is based on 10 replications, and for isolate 4 based on 5 replications.

2) 50 ml of supernate from the nematode culture added to each of the replicates.

3) Soil inoculated with 3000 nematodes and not planted.

added to the soil. The average number of nematodes recovered from soils inoculated with 500, 1500, and 3000 was 225, 965, and 2150, respectively. In the fallow soil inoculated with 3000 nematodes only 750 were recovered after the same period of time.

More individuals of *T. martini* were recovered from soil in which peas were growing than from fallow soil. Also, in soil inoculated with isolate 2 of *A. euteiches*, root rot was more severe in the pots infested with nematodes than in pots not infested with nematodes. These facts suggest that *T. martini* may parasitize the root of peas.

DISCUSSION

The fact that nematodes added to soil infected with the virulent isolate of *A. euteiches* increased the severity of root rot indicates that an association exists between the host, the fungus, and the nematode.

It has been postulated that the nematode aids root rot development by altering

the host physiology and by providing a favorable substrate for growth of the fungus by secreting enzymes that affect the host tissue or that create favorable avenues of entrance for the fungus pathogen.

As shown in the results, the severity of root rot increased when *T. martini* was used with the highly virulent isolate of *A. euteiches* and root rot severity increased proportionately to the increase in population of nematodes. When the nematodes were used with a weakly virulent isolate of *A. euteiches,* there was no increase in root rot severity. As there is little or no resistance to common root rot in commercial canning varieties, such as Perfected Wales, one might expect a greater increase in severity of root rot when the nematode was associated with the mildly virulent isolate than with the highly virulent isolate. Here apparently the nematode does not merely create more avenues of entrance but there is a more intimate relationship with a specific virulent isolate and/or the host plant. A similar association reported by Wyllie (1960) involves *Rhizoctonia solani* Kühn isolates with different degrees of virulence and the nematodes *Meloidogyne hapla* and *M. javanica.* Even though root rot development probably is dependent mainly on the virulence of the isolate and secondarily on the physiology of the host plant, more basic studies are necessary to ascertain the role of the nematode in the disease syndrome. Evidently there is some peculiar relationship between the host, the nematode, and a specific virulent isolate of the fungus, but the nature of this relationship remains to be explored.

ZUSAMMENFASSUNG

Der Einfluss parasitärer Nematoden auf die Stärke der Wurzelfäule bei Erbsen

Wurzelfäule bei Erbsen, die durch *Aphanomyces euteiches* verursacht wird, nahm in Gegenwart parasitärer Nematoden an Intensität zu. Der Nematode *Tylenchorhynchus martini* verstärkte das Ausmass dieser Krankheit selbst bei einer Populationsdichte von nur 500 Individuen pro 12,5 cm Tontopf. Die Wurzelfäule verschlimmerte sich nach der Zugabe der Nematoden nur dann, wenn mit einem stark pathogenen Stamm von *A. euteiches* gearbeitet wurde, wobei dieser Effekt in direkter Beziehung zu der Anzahl der zugegebenen Nematoden stand. Hingegen war in Gegenwart eines schwach pathogenen Stammes des Pilzes keine verstärkte Wurzelfäule zu beobachten. Die Anzahl der parasitären Nematoden, die vom Boden isoliert wurde, ergab eine direkte Korrelation zu der Anzahl der Nematoden, die in den Boden gebracht worden war. Es konnten nach derselben Zeitspanne aus den Töpfen, in denen keine Erbsen wuchsen, jedoch beträchtlich weniger Nematoden der Art *T. martini* isoliert werden als aus denen, die Erbsen enthielten.

REFERENCES

Caveness, F. E., & Jensen, H. J. (1955). Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. *Proc. helm. Soc. Wash.* **22**: 87-89.

Haglund, W. A., & King, T. H. (1959). The effect of nematodes on the development of root rot and yield of canning peas. *Plant Dis. Reptr* **43**: 787-790.

Johnson, H. G. (1953). Investigation on the control of root rot of canning peas. PhD. thesis, University of Minnesota.

Labruyere, R. E., Den Ouden, H., & Seinhorst, J. W. (1959). Experiments on the interaction of *Hoplolaimus uniformus* and *Fusarium oxysporum* f. *pisi* race 3 and its importance in "early yellowing" of peas. *Nematologica* **4**: 336-343.

Wyllie, T. D. (1960). Host-parasite relationships between soybean and *Rhizoctonia solani.* PhD Thesis, University of Minnesota.

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NEMATODE REACTIONS TO COAL-TAR DYES

BY

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The visual reactions of 28 genera of soil and plant nematodes to 38 coal-tar dyes fell into 3 classes: I — saturation reaction (primarily of order Enoplida), II — basophilic reaction (primarily of order Tylenchida), III — resistant reaction (most genera of orders Tylenchida and Rhabditida). Classes differed with respect to color, locus of staining and concentration thresholds. In general, both living and dead nematodes gave similar reactions to dyes.

Objectives were to study the reaction of nematodes to aromatic compounds and to develop techniques for investigation of the nematode cuticle. A general scheme of reaction classes and a report of basophilia (Singer, 1954) have been published (Hollis, 1960; 1961).

The nature of dye molecules made possible visual assessment of penetration, accumulation and persistence in the nematode, in addition to toxicity effects. Factors studied were dye solution concentration and pH, effects of killing and fixing, reversibility of staining and the killing and staining actions of iodine. The theory of staining and the chemical properties of basic and acid dyes have been described by numerous authors, including Conn (1946), Singer (1952) and Hill & Kelley (1932), while Cain (1947) has described properties and the action of nile blue sulfate on lipids and other substances.

Chitwood & Chitwood (1950) used intravitam stains to study cuticular permeability and found that marine nematodes possessed a more permeable cuticle than terricolous forms and that the cuticle of first stage larvae of rhabditoids and strongyloids was more permeable to stains than the later stages. Chitwood (1930) found that intravitam stains entered much more readily through the thin cuticle lining body openings. Weinstein (1960) has recently reviewed work on penetration of the nematode cuticle by dyes.

METHODS

Most nematodes (Table I) were maintained on host plants in the greenhouse, but a few were extracted from field samples. Species of *Rhabditis, Zeldia, Eucephalobus* and *Diplogaster* were propagated on bacteria in plates containing agar and *Dorylaimus ettersbergensis* de Man was propagated on a *Chroococcus* sp. Ten to fifteen unidentified species of *Dorylaimus* were tested.

The dyes used are listed in Table II. Nematodes were pipetted (0.5 ml) with 9.5 ml of dye solution in Syracuse watch glasses or 50 mm Petri plates and in-

TABLE I

Nematodes and their sources

Nematodes	Source [1])	Nematodes	Source [1])
Dorylaimus ettersbergensis de Man	Oats, Homer	*Helicotylenchus* spp.	Rice pasture, Crowley
D. pusillus Cobb [3])	Rice, Crowley	*Rotylenchus* sp.	Citrus, Port Sulfur
Dorylaimus spp.	Various	*Paratylenchus* sp.	Pine, Alexandria
Xiphinema americanum Cobb	Pine seedlings, Sibley	*Criconemoides* sp.	Pine, Alexandria
X. chambersi Thorne	Pine seedlings, Oberlin	*Hemicycliophora* sp.	Pine, Alexandria
Trichodorus christiei Allen	Cotton, Natchitoches	*Pratylenchus zeae* Graham	Corn, Homer
T. christiei	Florida [2])	*P. brachyurus* (Godfrey)	Corn, Homer
Discolaimus sp.	Various	*Radopholus oryzae*	
Mononchus sp.	Various	(v. Breda de Haan)	Rice, Kinder
Aphelenchoides besseyi Christie	Rice, Crowley	*Meloidogyne incognita*	
Aphelenchoides sp.	Coastal Bermuda grass, Homer	(Kofoid & White) larvae	Cotton, Shreveport
Tylenchorhynchus martini Fielding	Rice, Kinder	*Tylenchus* spp.	Various
T. acutus Allen	Corn, Homer	*Ditylenchus* spp.	Various
T. ewingi Hopper	Pine seedlings, Oberlin	*Psilenchus* spp.	Various
T. ewingi	Sugarcane, Baton Rouge	*Plectus* sp.	Rice, Kinder
Tylenchorhynchus sp.	Sugarcane, Grand Isle	*Rhabditis* spp.	Various
Tylenchorhynchus sp.	Oats, Homer	*Diplogaster* sp.	Various
Dolichodorus sp.	Oak, Alexandria	*Zeldia* sp.	Various
Belonolaimus longicaudatus Rau	Sugarcane, Grand Isle	*Cephalobus* sp.	Various
Hoplolaimus galeatus (Cobb)	Corn, Homer	*Eucephalobus* sp.	Various
		Acrobeloides sp.	Various

1) Crop and Louisiana town nearest collection site.
2) Supplied by Curtis Dieter, Dow Chemical Co., Winter Haven, Fla.
3) Identified by M. J. Fielding, E. I. du Pont de Nemours, Wilmington, Delaware.

cubated for 24 hours at 25-35° C. Dye solutions in distilled water were tested at concentrations ranging from 1 to 1000 ppm. Observations of nematode reactions in concentrated dye solutions (100-1000 ppm) were made after centrifuging and washing suspensions with distilled water.

TABLE II

Coal-tar dyes tested on nematodes

Name of dye [1]	Chromophore group	Dye reaction	Name of dye [1]	Chromophore group	Dye reaction
Naphthol green B	Nitroso	A [2]	Crystal violet	Phenyl methane	B
Picric acid	Nitro	A	Anilin blue w.s.	Phenyl methane	A
Martius yellow	Nitro	A	Fast green FCF	Phenyl methane	A
Congo red	Azo	A	Anilin green	Phenyl methane	B
Janus green B	Azo	B [3]	Brilliant green	Phenyl methane	B
Methyl orange	Azo	A	Hoffmann's violet	Phenyl methane	B
Aniline yellow	Azo	B	Rosanilin	Phenyl methane	B
Bismarck brown Y	Azo	B	Methyl violet 2B	Phenyl methane	B
Trypan blue	Azo	A	Methyl green	Phenyl methane	B
Chrysoidin Y	Azo	B	Light green SF	Phenyl methane	A
Brilliant cresyl blue	Quinone-imine	B	Eosin B	Xanthene	A
Toluidine blue O	Quinone-imine	B	Eosin Y	Xanthene	A
Nile blue sulfate	Quinone-imine	B	Rose bengal	Xanthene	A
Methylene blue	Quinone-imine	B	Acridine orange NO	Xanthene	B
Neutral red	Quinone-imine	B	Neutral acriflavine	Xanthene	B
Safranin O	Quinone-imine	B	Erythrosin, Bluish	Xanthene	A
Malachite green	Phenyl methane	B	Alizarin red S	Anthraquinone	A
Basic fuchsin	Phenyl methane	B	Primuline yellow	Thiazole	A
Acid fuchsin	Phenyl methane	B	Quinoline yellow	Quinoline	A

[1]) Nomenclature of dyes after Conn (1946).
[2]) A — acid.
[3]) B — basic.

Stains were tested in unbuffered solutions adjusted with HCl and NaOH, or in McIlvaine's citric acid-disodium phosphate buffer (Hodgman, 1949) and pH was determined electrometrically.

Iodine was mixed with potassium iodide, sublimated and dried over concentrated H_2SO_4. A saturated solution was prepared and its concentration was determined by thiosulfate titration. Solutions of known concentration was then prepared in screw-cap vials containing the test nematodes.

Roman numerals designate the three classes of nematode reactions and the degree of staining is shown by the code N — none, L — light, M — medium, H — heavy, V — very, P — partial (indicating that certain parts of the nematode took up stain).

RESULTS

The reactions of soil and plant nematodes to basic dyes fell into 3 classes: class I — saturation reaction (I), class II — basophilic reaction (II) and class III — resistant reaction (III).

TABLE III

Grouping of nematodes according to their reactions to basic dyes

Genera	Orders	Action of dye
Class I (stain readily)		
Dorylaimus, Discolaimus, Mononchus, Xiphinema, Trichodorus	Enoplida	L [1]
Diplogaster	Rhabditida	L
Class II (partially resistant to staining; cuticle stains but resists penetration of dye into body of nematode)		
Tylenchorhynchus, Aphelenchoides, Criconemoides, Tylenchus	Tylenchida	NL [2]
Rhabditis (juveniles)	Rhabditida	NL
Class III (resistant to staining)		
Plectus	Chromadorida	NL
Radopholus, Pratylenchus, Hoplolaimus, Helicotylenchus Rotylenchus, Dolichodorus, Belonolaimus, Paratylenchus, Ditylenchus, Psilenchus, Hemicycliophora, Meloidogyne (larvae)	Tylenchida	NL
Zeldia, Cephalobus, Eucephalobus, Acrobeloides	Rhabditida	NL

1) L — lethal, nematodes killed.
2) NL — nonlethal, nematodes living.

Class I — Saturation Reaction

This was observed in 5 genera of the Enoplida and in the genus *Diplogaster* of the Rhabditida (Table III). Both basic and acid dyes entered almost simultaneously at the front and rear of the body and moved gradually toward the center. Entry and spread of stain was sometimes confined to and generally more rapid at the front end. The color of staining was that of the dye solution. Polychrome dyes produced differential effects and dead specimens of *Dorylaimus* and *Xiphinema* species commonly exhibited a bright bluish-green esophagus and a dark-blue intestinal region containing pink fat globules. Eggs, if present, were rusty-red.

With the monochrome dyes neutral acriflavine, methyl green, brilliant cresyl blue or nile blue sulfate, as the adsorbed salt (Cain, 1947), the nematodes were saturated with dye, obscuring internal structures. Terminal dye concentrations appeared greater in the nematodes than in the solutions, and final stages of accumulation may have occurred against concentration gradients. All heavily-stained specimens died before the final stages of dye accumulation. Similarly, heavy staining occurred in heat-killed and formalin-preserved specimens.

The pattern of staining indicated dye entry through the cuticle at natural openings. Cuticular permeability, apart from natural openings, was demonstrated in several enoplidaceous genera by the diffusion of dye from undissolved particles through the cuticle at various points on the nematode surface. Entry at natural openings occurred at lower dye concentrations, probably because the cuticle is interrupted or is thinner in these regions (Chitwood 1930).

Death resulted from staining in concentrated solutions but basic dyes could be removed from the body by incubation of stained specimens in 0.1 N HCl at pH 1. Dorylaims varied in rates of destaining, requiring from 15 minutes to several hours, depending on degree of staining, species and dye.

Juvenile specimens of *Rhabditis,* which commonly exhibited reaction II, sometimes exhibited a combination of reactions I and II, with dye moving in from the anterior end. Reaction I was superposed on II, from which it was easily distinguished by differences in color, intensity and location of stain.

Reactions of Nematodes to Iodine

Soil and plant nematodes stained homogeneously with iodine, after the pattern of reaction class I, with primary entry occurring at the front and rear. Results of a typical iodine test are in Table IV. The minimum concentration required for staining nematodes under test conditions was 4 ppm. Enoplidaceous nematodes are more susceptible to killing by iodine and likewise to staining by dyes than other nematodes.

TABLE IV

Results of incubating of nematodes in iodine for 24 hours

Nematodes	Iodine (lethal conc.) range, ppm	Rating
Dorylaimus spp.	0.5—2	S [1]
Dorylaimus ettersbergensis	1—2	S
Trichodorus christiei	1—2	S
Tylenchorhynchus martini	2—4	R [2]
Radopholus oryzae	2—4	R
Zeldia sp.	2—4	R
Eucephalobus sp.	4—6	RR [3]
Plectus sp.	2—4	R

Visible staining occurred at 4 ppm and granulation of internal structures occurred at 6 ppm of iodine in 24 hours.

1) S — susceptible
2) R — resistant
3) RR — very resistant

Class II — Basophilic Reaction

Basophilic nematodes observed by the writer are in Table III. Uniform staining and limits on density in reaction II are due to localization of basic dye in the

cuticle. The characteristic violaceous tint of specimens stained with blue dyes probably results from metachromasy (Bergeron & Singer, 1958).

Visible staining of the cuticle of *T. martini* occurs with 1 ppm concentration of many basic dyes in 24 hours. In the laboratory, neutral acriflavine, a fluorescent dye, stained the cuticle of *T. martini* at 0.1 ppm in 24 hours, while methyl green was effective at 1 ppm.

Patterns of dye distribution were revealed by destaining of specimens in acid solutions and transfer to water followed by perforation of cuticle to permit emergence of internal contents.

Internal structures of nematodes in eaction class II were protected from dyes by the cuticle and stained partially only at high concentrations. Apparent resistance to staining increased in the ascending order of cuticle < esophagus < intestinal cell granules. Ability to retain dye (resistance to acid destaining) increased in the same order. The cuticle ordinarily destained in 30 sec., while the esophagus retained a faint stain reaction up to one-half hour in 0.1 N HCl. The cuticle of specimens could be destained and restained several times in a few minutes without impairing their viability.

Exposure of *T. martini* to dye concentrations of 100 ppm for one-half hour did not result in staining of internal structures. Incubation of specimens in 1000 ppm of dyes for 24 hours stained esophagus and intestinal granules. Figures 1 and 2 show stained intestinal cell granules in *T. martini* and *T. acutus,* after placing in 1000 ppm of nile blue sulfate for 24 hours then incubating in soil for 1 week. Retention of stain by intestinal granules occurred throughout the 3-month life of *T. martini* incubated for 1 week in soil then in water in the laboratory at room temperatures. Other structures, retaining dyes longer than the cuticle and esophagus, were the stylet knobs and basal part of the stylet shaft (meso- and metarhabdions) and the crescentic valve of the median esophageal bulb. Lines of the lateral field, particularly the outer ones, were prominent in stained specimens or those partially depleted of stain (Fig. 3, 4).

Juveniles of *Rhabditis* spp. were basophilic but adults commonly exhibited reaction I in concentrated dye solutions. The influence of age on stain reaction was evident in moulting specimens, with the partially-cast cuticle being basophilic and the new cuticle unstained in dilute solutions.

Staining of intestinal granules "rhabditin" occurred in *Rhabditis* spp. The retention of dyes by intestinal granules is not confined to nematodes exhibiting reaction II and occurred also in *T. christiei* and *Dorylaimus* spp.

Class III — Resistant Reaction

Reaction III resistance to basic dyes, at concentrations below and including 1000 ppm, is common in the Tylenchida and Rhabditida (Table III). Resistance to staining is correlated with resistance to dye toxicity and pH has little or no effect on the reaction.

Partial staining was common in reaction III. Genera of the Rhabditida showed staining of some intestinal cell granules and of areas in the esophagus. *Hemicycliophora* sp. stained lightly in clear areas of the esophagus and tail and in the male spicules.

Relative Reactions to Basic and Acid Dyes

The relative stainability of nematodes to basic and acid dyes in the pH range 6-8 was checked in a sample of percentage composition: Enoplida 18.8, Tylenchida 16.0 and Rhabditida 65.2. This sample, exposed 24 hours to 200 ppm of various dyes, gave the following percentages of total nematodes stained: (basic dyes) basic fuchsin 97.7, methyl green 86.2, neutral acriflavine 91.5; (acid dyes) acid fuchsin 21.7; eosin yellowish 4.8, rose bengal 6.0.

Most of the nematodes stained in the above experiment were in reaction class I. Responses of genera in reaction class III are in Table VI. Living *R. oryzae, Zeldia* sp., *Eucephalobus* sp. and *Plectus* sp. were uniformly resistant to both basic and acid dyes in the pH range 6-8.

Effect of pH on Nematode Reactions

Five dyes, 3 basic and 2 acid, were chosen for the initial experiment with nematodes from each stain reaction class (Table V). The depth of color of basic fuchsin, acridine orange NO, acid fuchsin and rose bengal varies with pH. Neutral red, an indicator of pH, is yellow in solutions more basic than pH 6.8. Shades of the fuchsins lighten with decreasing alkylation of the benzene rings brought about by increasing pH, (Conn, 1946). Solutions of acridine orange NO also became lighter with increasing pH.

Staining of *Dorylaimus* spp. became more intense with basic fuchsin and acridine orange NO (basic dyes) as pH increased, in spite of decreasing color intensity of the dye solutions. Likewise in rose bengal solutions, an acid dye that intensifies in color with increasing pH, the stain reactions of *Dorylaimus* spp. became more intense as pH decreased. Results with acid fuchsin were omitted because of bias introduced by pH effects on solution color intensity.

T. martini yielded a II reaction to basic dyes and a III reaction to the acid dye, rose bengal. *R. oryzae* gave a typical III reaction to both basic and acid dyes. There was some light-partial staining of this nematode in acridine orange NO at 1000 ppm concentration. Nematodes in classes II and III were unaffected by pH changes.

An additional experiment was conducted with acridine orange NO in McIlvaine's buffer at pH values ranging from 2.2 to 7.8 (data not shown). Little or no staining of *Dorylaimus* spp. occurred at pH 2.2, but intensity of stain increased gradually with pH and was medium to heavy at pH 7.8. Results with *Diplogaster* sp. were similar, although incomplete. Again, nematodes of classes II and III did not respond to pH changes.

Changes in staining intensity of *T. martini* with acridine orange NO were not detected at pH 2.2-7.8, but destaining was rapid in 0.1 N HCl at pH 1. Basophilism of this nematode was not reversed or removed by treatment with acid fuchsin in 0.1 N HCl. *T. martini, R. oryzae* and resistant genera of the Rhabditida were resistant to acid fuchsin in 0.1 N HCl, while genera of the Enoplida stained intensely.

TABLE V

Effect of pH on staining of nematodes [1] *for 24 hrs in basic and acid dyes at different concentrations*

Dye	Dye reaction	Solution conc. (ppm)	Solution [2] pH	*Dorylaimus* spp. (Class I)	*Tylenchorhynchus martini* (Class II)	*Radopholus oryzae* (Class III)
Basic fuchsin	Basic	50	4.2	I—LP-M	II—M	III—N
		50	7.0	I—M-H	II—M	III—N
		50	7.6	I—H	II—M	III—LP
Neutral red	Basic	50	7.0	I—L-M	II—L	III—N-LP
		50	7.2	I—L	II—L-M	
		50	7.8	I—LP-M	II—VL	
Acridine orange NO	Basic	50	4.0	I—L-M	II—L-M	III—N
		50	6.6	I—M-H	II—L-M	III—N
		50	7.1	I—M-H	II—L-M	
		50	7.3	I—M-H	II—L-M	
Acridine orange NO	Basic	1000	2.6	I—LP-L	II—M	III—LP
		1000	6.4	I—M	II—M	III—VLP
		1000	7.7	I—M-H	II—M	III—LP
		1000	8.8	I—H	II—M	III—LP
Rose bengal	Acid	100	3.6	I—H	III—N	
		100	6.9	I—M-H	III—N	
		100	7.7	I—LP-M	III—N	III—N
		100	8.0	I—L-M	III—N	
		100	8.3	I—M	III—N	III—N
Rose bengal	Acid	1000	3.3	I—H	III—N	III—N
		1000	7.5	I—M-H	III—N	III—N
		1000	8.4	I—M	III—N	III—N
		1000	9.7	I—L-M	III—N	III—N

[1] Legend: I — saturation reaction, II — basophilic reaction, III — resistant reaction; N — none, L — light, M — medium, H — heavy, V — very, P — partial.

[2] Dilutions were from 1000 ppm unbuffered solutions adjusted to different pH values with HCl and NaOH and determined electrometrically at the end of the experiment. Basic and acid fuchsin solutions lost some color with increasing pH, neutral red solutions were yellow at pH 7.2 and 7.8, acridine orange NO solutions lost color as pH increased and a light precipitate formed at pH 7.3, rose bengal solutions increased in color with increasing pH.

Reactions of Living and Dead Nematodes

Reactions to dyes of living nematodes and nematodes of the same sample killed by gentle heat and fixed in 10 % formalin were observed in 62 paired comparisons (Table VI). In 13/35 and 14/27 comparisons with basic and acid dyes respectively, there were slight increases in stain intensity due to killing and fixing. Killing and

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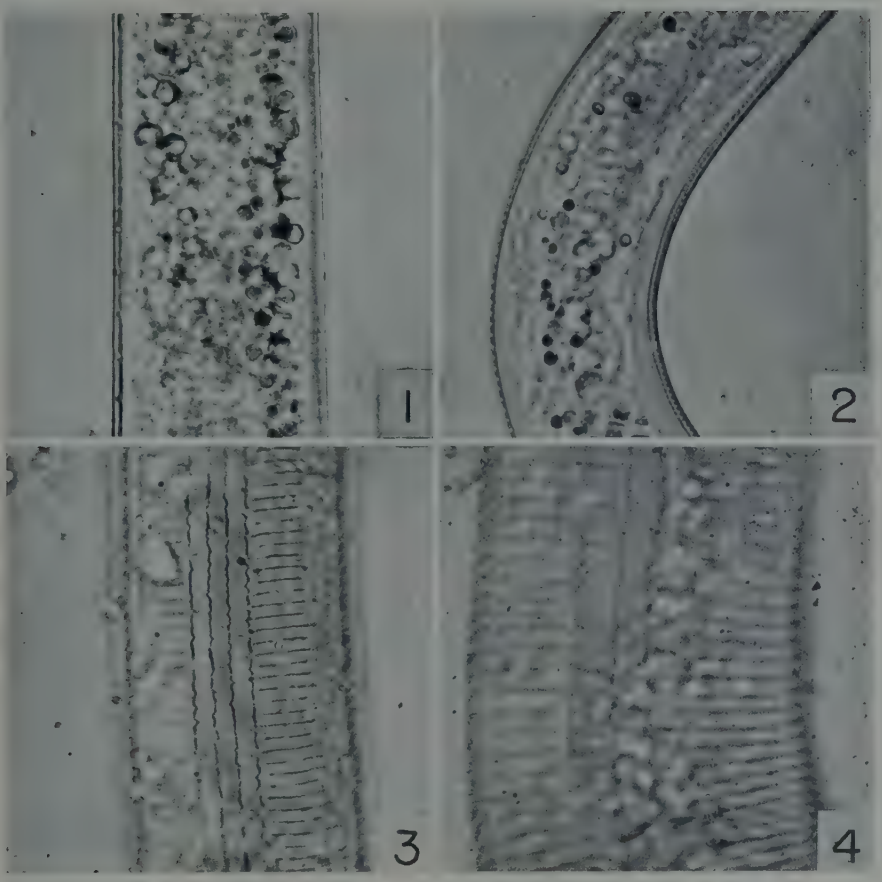

Fig. 1-4. Structures stained with nile blue sulfate (various concentrations). (1) Intestinal cell granules of *Tylenchorhynchus martini* (× 720). (2) Intestinal cell granules of *Tylenchorhynchus acutus* (× 625). (3) Lateral field of *Tylenchorhynchus martini* (× 900). (4) Lateral field of *Tylenchorhynchus ewingi* showing aerolation, after partial destaining (× 1500).

Table VI

Staining in basic and acid dyes for 24 hrs. of living, heat-killed and formalin-preserved nematodes (Solutions of 100 ppm at pH 6-8)

Dye	Dye reaction	Nematode status	*Dorylaimus* spp. [2]	*Trichodorus christiei*	*Tylenchorhynchus martini*	*Rhabditis* sp.	*Radopholus oryzae*	*Zeldia* sp.	*Diplogaster* sp.	*Eucephalobus* sp.	*Plectus* sp.
Toluidine blue O	Basic	Living	I—H	I—M-H	II—M	II—L	III—LP	III—L-MP	I—H	I—M-H	III—LP
		Dead	I—H	I—H	II—M	II—L-M	III—LP	I—M	I—H	I—M-H	I—M-H
Methylene blue	Basic	Living	I—H	I—M-H	II—M-H	II—L	III—LP	III—L-MP	I—H	I—H	III—LP
		Dead	I—H	I—M-H	II—M-H	II—L	III—L-MP	I—H	I—H	I—M-H	I—M
Basic fuchsin	Basic	Living	I—M-H	I—M-H	II—L-M	II—L	III—LP-L	III—N-LP	I—H	III—L	
		Dead	I—H	I—M-H	II—L-M	II—L	III—LP	I—M-H	I—H	I—H	I—M
Acridine orange NO	Basic	Living	I—M-H	I—M	II—L	II—L	III—N	III—N-L	I—M	I—M	III—N-L
		Dead	I—M-H	I—M	II—L	II—L	III—LP	I—L-M	I—H	I—L-M	III—L
Acid fuchsin	Acid	Living	III—N-VLP	III—N-VLP	III—N	III—N	III—N	III—N	III—N	III—N-VLP	III—N
		Dead	I—LP-M	III—VL	III—N-L		III—N	III—VL	III—L	III—VL	III—N
Congo red	Acid	Living	III—N-LP	III—N	III—N	III—N-LP	III—N	III—N-LP		III—N	III—L
		Dead	III—N-LP	III—N	III—N	III—N-VLP	III—N		III—LP	III—N	
Eosin Y	Acid	Living	III—MP	III—VLP	III—N	III—N	III—N	III—VLP			
		Dead	III—VL	III—VL	III—N-L	III—N	III—N	III—VL	III—VL	III—VL	III—N
Rose bengal	Acid	Living	I—L-M	III—N	III—N	III—N	III—N-LP	III—VLP	III—N	III—N	
		Dead	I—M	III—VLP	III—N-VL	III—MP	III—VLP	III—VL	III—LP		III—LP

[1]) Legend: I — saturation reaction, II — basophilic reaction, III — resistant reaction; N — none, L — light, M — medium, H — heavy, V — very, P — partial.

[2]) Living nematodes killed by the basic dyes were *Dorylaimus* spp., *Trichodorus christiei* and *Diplogaster* sp. Acid dyes had no effect on these genera. All other nematodes were alive and motile in all dye solutions. The breaking of resistance to the 100 ppm concentrations by killing and fixing is indicated by a change from Class III to Class I notation.

fixing resulted in 7/35 cases of breaking resistance to basic dyes at 100 ppm, from a class III to a class I reaction, and only one instance of breaking resistance with acid dyes. In general, the integrity of the different reaction classes was maintained in killed and preserved nematodes under the experimental conditions.

DISCUSSION

Results with enoplidaceous nematodes support certain observations of Filipjev (1934) and Chitwood (1930). Effects of pH of basic and acid dye solutions on these nematodes suggest an isoelectric range on the acid side of neutrality for the cuticle and a preponderance of negatively-charged groups at staining sites.

Stainability of nematodes in the different reaction classes correlates with susceptibility to iodine and to halogenated hydrocarbons (Hollis & Fielding, 1958). This suggests that relative reaction of different groups of nematodes to nematocides is a function of cuticular permeability rather than dosage tolerance.

Iodine, picric acid and aniline yellow stain all soil and plant nematodes in a manner similar to reaction class I. Mechanisms permitting these substances to bypass, modify or eliminate reaction III resistance are of interest.

The results of this work indicate that coal-tar dyes comprise useful tools, in terms of cadre and discipline, for investigations of the nematode cuticle.

ZUSAMMENFASSUNG

Nematodenreaktionen auf Kohlenteerfarbstoffe

In verdünnten wässrigen Lösungen wurden Färbungs- und Sterblichkeitsreaktionen von 28 Gattungen boden- und pflanzenbewohnender Nematoden auf basische und saure Kohlenteerfarbstoffe studiert. Die Reaktionen der Nematoden auf die basischen Farbstoffe wurden in drei verschiedene Klassen aufgeteilt. Klasse I — die Sättigungsreaktion (1) — wurde an 5 Gattungen der Enopliden und an der Gattung *Diplogaster* (Rhabditida) beobachtet. Die Minimal-Farbstoffkonzentrationen für die Färbung und für die Sterblichkeit der Nematoden in I reichten in der Zeit von 24 Stunden von 10 bis 1000 ppm, was in erster Linie durch die Art der Nematoden bedingt ist. Klasse II — basische Reaktion (II) — wurde an 4 Gattungen der Tylenchida und an Jungtieren der Gattung *Rhabditis* (Rhabditida) gezeigt. Die Nematoden, die die Reaktion II gezeigt haben, reagierten innerhalb von 24 Stunden stark auf die Färbung der Oberflächenhaut mit Minimal-Farbstoffkonzentrationen. Sie widersetzten sich aber einer tieferen Färbung, und Sterblichkeit trat nicht ein unter Farbstoffkonzentrationen bis zu 1000 ppm. Die Verschiedenartigkeit der beiden Effekte (Färbungs- und Sterblichkeitseffekt) hing hauptsächlich von dem Farbstoff ab. Klasse III — Resistenz-Reaktion — eine Reaktion, bei der die Färbung nur wenig oder gar keinen Erfolg hatte, wurde in 12 Gattungen der Tylenchida, in 4 Gattungen der Rhabditida und in der Gattung *Plectus* (Chromadorida) gefunden. Die geprüfte Maximal-Farbstoffkonzentration der Klasse III wurde innerhalb von 24 Stunden willkürlich auf 1000 ppm festgestellt. Die größere Reaktionsfähigkeit der Nematoden auf basische im Vergleich zu saueren Farbstoffen in I und II beweisen, daß die gefärbten Stellen ein Übermaß negativ-geladener Gruppen tragen. I war, der Färbungstheorie gemäß, pH-abhängig, während II and III unabhängig von pH waren. Die Integrität der Reaktionsklassen bleibt hauptsächlich bei Fadenwürmern erhalten, die durch Hitze getötet und in Formalin aufbewahrt wurden.

Die Reaktionen aller Nematoden auf Jod gehören zum Typus I. In allen Nematoden waren die Minimal-Konzentrationen von Jod, die die Färbungs- und Sterblichkeitseffekte in 24 Stunden verursachten, 4 ppm und 0.5-6 ppm. Es gab Wechselbeziehungen zwischen den Färbungsreaktionen der verschiedenen Nematoden auf basische Kohlenteerfarbstoffe und ihren Sterblichkeitsreaktionen auf Jod.

REFERENCES

BERGERON, J. A. & SINGER, M. (1958). Metachromasy. An experimental and theoretical reevaluation. *J. Biophys. Biochem. Cytol.* **4**, 433-457.

CAIN, A. J. (1947). The use of nile blue sulfate in the examination of lipids. *Quart. J. micr. Sci.* **88**, 383-392.

CHITWOOD, B. G. (1930). Studies on some physiological functions and morphological characters of Rhabditis (Rhabditidae, nematodes). *J. Morph.* **49**, 251-275.

CHITWOOD, B. G. & CHITWOOD, M. B. (1950). *An introduction to Nematology.* B. G. Chitwood. Baltimore, Md. 213 pp.

CONN, H. J. (1946). *Biological stains.* 5th Ed. Biotech, Publ., Geneva, N.Y. 346 pp.

FILIPJEV, I. N. (1934). Classification of the free-living nematodes and their relation to the parasitic nematodes. *Smithson. misc. Coll.* **89**: (6): 1-63.

HILL, G. A. & KELLEY, L. (1932). *Organic chemistry.* Philadelphia, Pa. P. Blakiston's Son and Co., Inc. 564 pp.

HODGMAN, C. D. (Editor) (1959). *Handbook of Chemistry and Physics,* 31st. Ed. Cleveland, Ohio. Chem. Rubber Publ. Co. 2737 pp.

HOLLIS, J. P. (1960). Reactions of soil nematodes to coal-tar dyes. *Nature, Lond.* **188**, 1128-1129.

—— (1961). Basophilia of soil and plant nematodes. *Phytopathology* **51**, (Abstr.).

HOLLIS, J. P. & FIELDING, M. J. 1958. Population behaviour of plant parasitic nematodes in soil fumigation experiments. *Bull. La agric. Exp. Sta.* 515, 30 pp.

SINGER, M. (1952). Factors which control the staining of tissue sections with acid and basic dyes. *Int. Rev. Cytol. 1952,* 211-255.

—— (1954). The staining of basophilic components. *J. Histochem. Cytochem.* **2**, 322-333.

WEINSTEIN, P. P. (1960). Excretory mechanisms and excretory products of nematodes: an appraisal, pp. 65-92. In: STAUBER, L. A. (ed.), *Host influence on parasite physiology.* Rutgers Univ. Press, New Brunswick, N. J.

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THE USE OF NEMATODE-TRAPPING FUNGI TO CONTROL ROOT-KNOT NEMATODES

BY

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A series of experiments was conducted to determine whether root-knot disease of tomato and okra (*Hibiscus esculentus*) could be controlled by the use of nematode-trapping fungi. The fungi did not reduce disease severity; however, three species significantly affected survival of plants in a greenhouse experiment. Immediate protection of crops highly susceptible to root-knot nematodes by the application of predacious fungi does not seem possible.

Experiments to control root-knot nematodes by predacious fungi were first made in Hawaii by Linford & Yap (1939) after Linford, Yap & Oliveira (1938) had shown that marked reductions in root-knot nematode populations occurred during decomposition of plant materials mixed with infested soil. Eleven nematode-trapping fungi were recognized among 52 natural enemies of the root-knot parasite. They tested the abilities of five predacious hyphomycetes, *Arthrobotrys oligospora* Fres., *A. musiformis* Drechs., *Dactylella ellipsospora* Grove, *Dactylaria candida* Sacc., and *D. thaumasia* Drechs., to protect potted pineapple plants from the root-knot nematode. Only *Dactylella ellipsospora* restricted nematode injury somewhat; the others were ineffective.

Deschiens, Lamy & Vautrin (1943) used *Dactylella bembicodes* Drechs. and *Arthrobotrys oligospora* to protect Begonia cuttings. The cuttings were grown in pots of infested soil plunged in a bed of peat also infested with root-knot nematodes. Spores of the fungi were added to 21 pots, while 18 others were left as untreated controls. Of the 21 inoculated pots, ten received *A. oligospora* and eleven *D. bembicodes*. The plants were grown for about 6 months and then examined for root-knot galls. Results for individual plants were not given; however, three of the 21 treated plants were infected (14 %) with a total of five galls observed; eight of the eighteen control plants were infected (44 %) with a total of 85 galls observed on the roots. Although the authors state that the results show an important level of protection due to the addition of the fungi, Duddington (1957) has indicated that the results are not statistically significant.

Mankau (1961) attempted to reduce root-knot damage to tomato and okra by inoculating the predacious hyphomycetes, *Dactylaria thaumasia* and *Arthrobotrys arthrobotryoides* (Berl.) Lindau, into soil infested with *Meloidogyne incognita*

Chitwood, but was unsuccessful. Soil treatments of wheat germ, straw, and steer manure used in conjunction with the two fungus species gave better plant growth, but did not appear to increase the activity of the fungi.

This report presents the results of further attempts to measure reduction in plant injury by *Meloidogyne incognita* using other predacious fungi under varied circumstances.

METHODS AND MATERIALS

The nematodes used originated from greenhouse stock cultures obtained from single egg mass transfers of *Meloidogyne incognita* and subcultured on suitable hosts, usually tomato.

The fungi were grown in broth culture in large medicine bottles incubated horizontally to provide shallow depth and a large surface area suitable for sporulation. The broth medium was prepared by extracting either sugar beet pulp or wheat germ at the rate of 20 g per liter of water for 10 minutes in an autoclave and then dispensing it to culture bottles and autoclaving for 15 minutes at 1.03 kg per sq. cm (15 p.s.i.). Mycelial mats were recovered after 2 to 3 weeks by pouring cultures onto filter paper in a Büchner funnel and separating the broth. The mats were then macerated in distilled water for 30 sec. Each batch of inoculum was checked for viability before use by plating a loopful on corn meal agar. The inoculum was injected into the soil of test pots or microplots with a 50-ml steel syringe.

EXPERIMENTAL

In two preliminary experiments to determine the effect of predacious fungi upon a root-knot nematode in the absence of most of the normally associated soil flora and fauna, plants were grown in quartz sand in 1.5-liter plastic containers furnished with lids having a pour-spout and a hole for the plants. The spaces around the stems were closed with glass wool. The plants were irrigated with Hoagland's solution introduced through the capped pour-spout which was opened briefly to minimize aerial contamination.

In the first experiment tomato seedlings (var. Rutgers) and the fungi *Arthrobotrys conoides* and *A. dactyloides* were used. Five egg masses of *M. incognita* were introduced per container. Three treatments were applied: nematodes only, nematodes and fungi, untreated checks. Replication was fourfold.

Fresh top weights recorded at the end of the 90 days' growth (Table I) revealed no significant differences due to the treatments. The root systems of the plants were washed from the sand, examined and scored for root-knot infestation. Plants in the treatment receiving the predacious fungi were as heavily galled as those in the series receiving only nematode inoculum. *A. dactyloides* was recovered from each inoculated container, while *A. conoides* was recovered from two of the four containers.

TABLE I

Fresh top weights of tomatoes in sand medium and closed containers (grams)

Treatment	Replicates 1	2	3	4	Average
A Nemas only	254	305	276	202	259.2
B Nemas + fungi	230	304	296	281	277.7
C Control	264	338	234	264	275.0

Relative severity of galling in treatments A and B:

Treatment	1	2	3	4
A	O	O	H	H
B	L	H	H	M

O = no galls observed, L = light, M = medium, H = heavy infestation.

In the second experiment okra (*Hibiscus esculentus*) was used, and the treatments included *Arthrobotrys arthrobotryoides*, *A. dactyloides*, *Dactylaria thaumasia*, *D. brochopaga* and *Dactylella ellipsospora*, each replicated twelve times. Six containers (i.e., half the replicates) of each treatment were inoculated before addition of nematodes (830 larvae/container) and six after addition. For controls, six containers were inoculated with nematodes only and six received neither nematodes nor fungi.

The plants grew so poorly that the experiment was terminated after 3 months. It was impossible to measure accurately the effects of the treatments on the plants, but root-knot developed in all inoculated plants, and larvae and males occurred in all containers at the end of the test except in uninoculated controls. All inoculated fungi were recovered from the respective treatments, but no effect upon the remaining nematode population could be detected.

A greenhouse experiment incorporating all six of the previously mentioned fungi was made to study their ability to protect a crop from root-knot damage in a more nearly normal soil condition. The experiment was done in two types of soil, one steam-sterilized only and the other steam-sterilized to which sugar beet pulp was added at the rate of 250 cc to each 3-gal. glazed pot. The pots were planted with tomato seedlings and a week later were injected with the appropriate fungus. A few days later 2,000 *M. incognita* larvae were added in water suspension. Pots with nematodes but no fungi and with neither were set up for both soils. Replication was sixfold and the pots were set out on the bench in a simple randomized block layout.

At the end of the test, plants were scored on the basis of *0* for a dead plant, *1* for an obviously dying plant, and *2* for a living plant. The addition of fungi

extended the survival of plants to a statistically significant degree in three instances (Table II) where *D. ellipsospora* and *A. conoides* were added to treated soil and where *D. thaumasia* was added to nontreated soil. The treatments had no significant effect on the larval population of the pots.

TABLE II

Effect of nematode-trapping fungi on the survival of root-knot infested tomato plants

Treatments	Sugar-beet pulp	Total plant scoring	Mean	Average larval count/50 cc soil
A. arthrobotryoides	—	0	0	15
	+	2	0.3	4
D. brochopaga	—	3	0.5	15
	+	3	0.5	10
A. dactyloides	—	0	0	10
	+	1	0.2	14
D. thaumasia	—	6	1.0*	42
	+	3	0.5	11
D. ellipsospora	—	0	0	20
	+	7	1.2*	7
A. conoides	—	0	0	21
	+	6	1.0*	19
Fungus check	—	0	0	18
	+	1	0.2	15
Nematode-free check [2]	—	(11) [1]	1.8	(0)
	+	(10)	1.7	(0)

[1] Less than possible score of 12 because of effects of virus disease apparently present in all plants.
[2] Excluded from Analysis of Variance.
* Significantly different from both checks at 5 % level. $s_{\bar{x}} = 0.26$.

Soil from each pot was also plated on weak corn meal agar to determine which predacious hyphomycetes were present. Inoculated fungi were not recovered from some of the pots into which they had been introduced, and a rather widespread occurrence of contamination in the pots by some of the fungus species was observed. Care was taken to avoid splashing while watering the pots, but the contamination displayed a pattern which was very likely caused by watering. Of the six species of fungi used, only 3 occurred as contaminants: *A. conoides*, *A. arthrobotryoides*, and *D. ellipsospora*. The other species, *D. brochopaga*, *D. thaumasia* and *A. dactyloides*, were found only in pots in which they had been inoculated, with one exception, *A. dactyloides*, which occurred in an adjacent pot as a contaminant.

Outdoor microplot test

Two fungi, *Arthrobotrys dactyloides* and *Dactylella ellipsospora* were used in

an outdoor microplot test. A population of *M. incognita* had been built up in the soil by previous croppings. The area was a 4.5 m × 9 m cubicle bordered by steel plate sunk approximately 1.5 m into the soil, and contained sixteen circles of galvanized sheet steel 1 m in diameter sunk approximately 30 cm into the soil. Three tomato seedlings were transplanted into each microplot and the soil between the circular microplots was fallowed.

Eight of the sixteen microplots were inoculated with the predacious fungi, and the remaining eight served as untreated controls. Four of the fungus-inoculated plots received inoculum prepared in broth culture in the laboratory (treatment A), while the other four received 1.5 g of powdered dry spores of the same fungus isolates prepared by a commercial laboratory and mixed into the soil at the base of each plant (treatment B). The treatments were randomized in the microplots.

The plants were grown for 110 days and then harvested by cutting the stems at ground level and measuring fresh top weights. The root-knot larvae population in the soil in each plot was examined by combining ten cores from each plot taken with a 2.5 cm diameter soil-sampling tube to a depth of about 25 cm and washing and sieving 50 g of the sample to remove the nematodes. The results are given in Table III.

Substantial galling of the tomato roots occurred in all plots. Although plants in treatment B appeared to have a better average growth, the differences between treatments were not statistically significant. Larval populations in the soil were highest in B, and average numbers of larvae in fungus-treated plots differed only slightly from untreated plots.

TABLE III

Outdoor microplot test.
Fresh top weights and counts of root-knot larvae/50 g soil.

Treatment		Replicates 1	2	3	4	Average
A. Fungi-laboratory broth	Top wt. (g):	963	841	1033	335	793
	Larvae:	(1350)	(440)	(320)	(230)	(585)
B. Fungi-commercial powdered spores	Top wt. (g):	2200	1708	674	198	1195
	Larvae:	(1130)	(2130)	(1400)	(820)	(1370)
C. Control (1-4)	Top wt. (g):	575	331	550	957	
	Larvae:	(60)	(660)	(560)	(120)	
						(8 replicates)
						680
						(875)
Control (5-8)	Top wt. (g):	437	806	766	1021	
	Larvae:	(300)	(130)	(560)	(1110)	

Soil from each plot was plated directly on weak corn meal agar in Petri dishes and observed over a period of 41 days. *Arthrobotrys oligospora* was observed from one of the control plots, and *D. ellipsospora* occurred in a plate from another.

Stylopage leiohypha, a predacious member of the Zoopagaceae, occurred in every plot. The inoculated fungi, therefore, were not reisolated from the soil at the end of the test.

DISCUSSION

The results of this series of tests indicate that the introduction of predacious fungi into soil to protect tomato and okra from attack by root-knot larvae in infested soil was unsuccessful. However, different nematode-trapping fungi evidently vary in their effect upon a root-knot infestation, as seen from the greenhouse test of a number of species. This test also indicated that factors such as soil type or organic content may influence the activity of predacious fungi. Nevertheless, none of the fungi tested were able to prevent serious damage to the hosts of root-knot nematodes used in these tests.

Both okra and tomato used in these tests and in the earlier report (Mankau, 1961 b) produce fleshy root systems which become heavily galled when attacked by root-knot nematodes. Under such circumstances repeated infestations commonly occur in the roots with the result that the larval progeny of a single nematode may multiply within a large gall where predacious fungi cannot reach them. Linford & Yap (1939) reported the effect of some of the fungi used in the present study in protecting pineapple plants from a root-knot infestation. Pineapple has a fibrous root system in which the egg masses of mature females may be exposed on the surface of the roots and in which it is rare for larvae to re-enter the same gall. Yet even in this type of host, only one fungus, *D. ellipsospora*, out of five species tested gave a beneficial response. This species was one of three which gave a detectable, though inadequate, protection in this study. Predacious fungi are probable ineffective against root-knot infestations within such favorable hosts as tomato and okra in which the reproductive potential of the parasite is very great. The study throws no light on the capabilities of such fungi during decomposition of host roots and larval migration in the soil.

The contamination of treatments by predacious fungi other than those inoculated presents a problem which must be considered in evaluating the effect of certain species. The faster-growing, more heavily-sporulating species were less frequently contaminants. An ephemeral activity by an abundance of inoculum of a species which quickly declines or disappears may also influence the outcome of a test and yet have little practical application in nature. It would appear necessary that any claim of control of a nematode pest by an introduced biological agent should be substantiated by demonstrating that the organism used could be reisolated from the environment, or that it was present long enough to have controlled the pest.

In some instances the fungi added to soil could not be recovered at the end of a test. They were more easily recovered from the sterilized sand media than from either steamed or natural soil. Attempts by the writer to establish predacious fungi in soil have given variable results and were often unsuccessful. This sug-

gests that these fungi are in competition with antagonistic elements of the flora and fauna of soils (Mankau, 1961 a). Even when inoculated fungi were recovered, it could not be determined whether they existed in the soil as dormant conidia or actively trapping mycelia.

All of the fungi used here and by previous investigators against root-knot nematodes are effective in decimating a population of larvae in agar culture in a matter of a few days. They may play an important role in soils in which they occur and some species are rather widespread (Drechsler, 1941; Duddington, 1957; Mankau & Clarke, 1959; and Shepherd, 1956). However, relatively little is known of these organisms under natural conditions, and their activity within the soil is difficult to assess. Although it may be possible to devise methods to utilize them effectively, our present knowledge is insufficient to enable use of them to be made in the control of plant-parasitic nematodes. Van der Laan (1956) came to a similar conclusion in his studies on biological control of *Heterodera rostochiensis.*

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ZUSAMMENFASSUNG

Die Verwendung nematodenfangender Pilze zur Bekämpfung von Wurzelgallenälchen

Einige Versuche wurden durchgeführt, um festzustellen, ob ein Befall von Tomaten und Okra (*Hibiscus esculentus*) mit Wurzelgallenälchen, *Meloidogyne incognita* Chitwood, durch die Verwendung verschiedener Arten räuberischer Pilze in einer Test-Serie zu bekämpfen ist. Die Pilze konnten den Befall der Pflanzen nicht eindämmen, jedoch zeigten Versuche im Glashaus, dass einige Arten räuberischer Pilze das Leben infizierter Pflanzen auffallend verlängerten. Die Anwendung von Impfungen in der Erde zum direkten Schutz der Pflanzen vor Befall durch Wurzelgallenälchen wird nicht für zweckmässig gehalten.

REFERENCES

Deschiens, R., Lamy, L. & Vautrin, E. (1943). Essais pratiques de prophylaxie de l'anguillulose des végétaux par l'emploi d'hyphomycétes prédateurs, *Comp. Rend. Acad. Sci. Paris* **216**, 539-541.

Drechsler, C. (1941). Predacious fungi. *Biol. Rev.* **16**, 265-290.

Duddington, C. L. (1957). *The Friendly Fungi.* London, Faber & Faber, Ltd. 188 pp.

Laan, P. A. van der (1956). Onderzoekingen over schimmels, die parasiteren op de cysteinhoud van het aardappelcystenaaltje (*Heterodera rostochiensis* Wollenweber) *Tijdschr. PlZiekt.* **62**, 305-321.

Linford, M. B. & Yap, Francis (1939). Root-knot nematode injury restricted by a fungus. *Phytopathology* **29**, 596-609.

Linford, M. B., Yap, Francis & Oliveira, Juliette M. (1938). Reduction of soil populations of the root-knot nematode during decomposition of organic matter. *Soil Sci.* **45**, 127-140.

Mankau, R. (1961a). Antagonisms to nematode-trapping fungi in soil. *Phytopathology* **51**, 66. Abs.

Mankau, R. (1961b). An attempt to control root-knot nematode with *Dactylaria thaumasia* Drechsler and *Arthrobotrys arthrobotryoides* Lindau. *Plant Dis. Deptr* **45**, 164-166.

Mankau, R., & Clarke, O. F. (1959). Nematode-trapping fungi in southern California citrus soils. *Plant Dis. Reptr* **43**, 968-969.

Shepherd, A. M. (1956). A short survey of Danish nematophagous fungi. *Friesia* (Copenhagen) **5**, 396-408.

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NEMATODES ATTACKED BY PROTOZOA AND TARDIGRADES

BY

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Observations were made on a ciliate protozoon, *Urostyla* sp., ingesting live nematodes but apparently unable to digest them. Tardigrades, *Macrobiotus* sp., were also observed attacking and feeding on nematodes.

Amoeboid protozoa are known to attack nematodes (Weber, Zwillenberg & Van der Laan, 1952; Winslow & Williams, 1957), and Doncaster (1956) reported a ciliate protozoon, *Stylonichia pustulata*, ingesting moribund nematodes. Recently another ciliate protozoon, *Urostyla* sp. was observed ingesting live nematodes.

Unidentified tardigrades have been reported attacking nematodes, (Hutchinson & Streu, 1960) and we have observed *Macrobiotus* sp., attacking and feeding on nematodes.

Ingestion of nematodes by protozoa (Fig. 1).

Many individuals of the ciliate protozoon *Urostyla* sp. and many nematodes (*Rotylenchus, Tylenchorhynchus* and saprobes) were extracted from a pot of soil in which *Agrostis tenuis* had grown for a year. A water suspension of the soil was sieved and organisms in the residue were allowed to filter through a cotton wool pad into clean water overnight. The following morning many *Urostyla,* measuring about 300 μ in length, were seen to ingest nematodes up to nearly 1 mm long. The smaller ones were sometimes completely ingested, but rarely more than about a third of the body length of the largest nematodes was taken into the *Urostyla.* The large ones were usually soon dropped and showed no ill effects, except that a deposit resembling mucus adhered to the cuticle and to it particles of debris became attached. However, either the head or the tail of the nematode, whichever was ingested first, often gradually protruded from the side or aboral end of the *Urostyla* until the protozoon pellicle was finally ruptured. For at least several minutes the protozoa, thus damaged, continued to swim about with undiminished activity, the nematode usually adding an undulatory movement of its own.

A few *Urostyla* were found containing a nematode coiled up and motionless, protruding nowhere. In an attempt to find out whether nematodes were digested, eight *Urostyla* completely, or almost completely enclosing nematodes, were pipet-

ted into a watch glass and re-examined after 3 hrs. Six nematodes had escaped and were apparently unaffected and two *Urostyla* still contained their prey. Two of the *Urostyla* had disappeared. While under observation one or two *Urostyla* each containing a large, active nematode were seen suddenly to rupture and then disintegrate, and it seems probable that this may be common.

After 48 hrs in the presence of active nematodes, most of the *Urostyla* had become encysted.

Ten freshly extracted *Urostyla* from the same source were placed in 5 ml of water in a watch glass, together with eggs, larvae and adults of *Ditylenchus destructor* and some potato starch grains. It was not until about 2 hrs later that the *Urostyla* began to ingest both nematodes and starch, but after 18 hrs two of the ciliates had disappeared, seven had encysted and only one was still active.

Populations of this *Urostyla* sp. apparently cannot survive on nematodes and this protozoon should not be regarded as a predator of nematodes, even though it may ingest them in certain circumstances.

Tardigrades as predators of nematodes (Fig. 2).

Le Gros (1958) states that tardigrades sometimes attack nematodes in addition to rotifers and other tardigrades, and Hutchinson & Streu (1960) reported unidentified tardigrades attacking nematodes in the U.S.A. and Ceylon.

In October, 1960, tardigrades and nematodes were extracted together from samples of turf from Swansea, Wales by a technique similar to that described in the preceeding section. From the key given by Le Gros (1958) the tardigrades were identified as *Macrobiotus* sp.

Although the tardigrades could not move about very rapidly in the water extract, a great many succeeded in fastening on to nematodes moving near them. They showed no preference for size of prey. Distinct tears were made in the nematode cuticle by the action of the tardigrades' oral stylets. Nematodes reacted vigorously for a time, but they eventually became immobile whether or not the tardigrade was still attached. It seemed possible that a toxic secretion had been passed into the nematode.

Tardigrades could maintain a hold even on large, vigorously moving nematodes such as monochids, and, from examination of dead and preserved specimens, the nematode seemed to be drawn partly into the mouth of the tardigrade by suction when the nematode was held transversely or with one end enclosed in the tardigrade's mouth tube. Signs that some of the body contents of the nematode had been withdrawn by the tardigrade were often seen and many nematodes had more than one tear in their cuticle. Occasionally, two tardigrades were seen attached to one nematode and a moribund enchytraeid was fed on extensively by at least six tardigrades.

Ciné film records were made of *Urostyla* ingesting nematodes and of *Macrobiotus* preying on nematodes.

Fig. 1. *Urostyla* sp. ingesting nematodes: A, nematode partly ingested and affecting the body shape of the *Urostyla*. B, left, nematode partly ingested; right, nematode completely ingested. C, right, *Urostyla* with partly ingested nematode; left, *Urostyla* showing damage caused by an ingested nematode. D, *Urostyla* with one end of nematode protruding from the oral groove and the other end through the ruptured pellicle. (A, high speed flash photo; B-D, stills from ciné film).

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Fig. 2. Tardigrades, *Macrobiotus* sp., attacking nematodes: A, nematode attacked by tardigrade. B, nematode with tardigrade attached, also showing cuticular tears (arrowed) caused by previous attacks. C, body contents of nematode being withdrawn by attacking tardigrade. D, nematode partly drawn into mouth tube of tardigrade. (A-C, stills from ciné film; D, photo of lacto-phenol mount).

Macrobiotus seemed well able to survive for some days on a carnivorous diet including live nematodes, and it is probably a real predator of nematodes and other fauna in the soil.

The authors thank Dr J. Meiklejohn for identifying the ciliate protozoon *Urostyla* sp.

ZUSAMMENFASSUNG

Nematodenbefall von Protozoen und Tardigraden

Nematoden in einem Wasserextrakt aus einem Rasenstück wurden von einem Einzeller, *Urostyla* sp. gefressen. Gelegentlich entweichen die Nematoden, ohne anscheinend Schaden genommen zu haben. Zuweilen war *Urostyla* geschädigt. Tardigraden, *Macrobiotus* sp., griff Nematoden gern an und frass an ihnen in einem Wasserextrakt aus Boden. Unter Umständen trat der Tod bei den Nematoden ein.

REFERENCES

Doncaster, C. C. (1956). Electronic flash in photomicrography. *Nematologica* **1**, 51-55.

Hutchinson, M. T. & Streu, H. T. (1960). Tardigrades attacking nematodes. *Nematologica* **5**, 149.

Le Gros, A. E. (1958). How to begin the study of tardigrades. *Countryside* **18**, 322-330.

Weber, A. Ph., Zwillenberg, L. O. & Van der Laan, P. A. (1952). A predacious Amoeboid organism destroying larvae of the potato root eelworm and other nematodes. *Nature Lond.*, **169**, 834.

Winslow, R. D. & Williams, T. D. (1957). Amoeboid organisms attacking larvae of the potato root eelworm (*Heterodera rostochiensis* Woll.) in England and the beet eelworm (*H. schachtii* Schm.) in Canada. *Tijdschr. Plziekt.*, **63**, 242-243.

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SHORT COMMUNICATION

D. J. HOOPER [1]). *A method for attaching the funnel to the flask for use in the Seinhorst two-Erlenmeyer-flask soil extraction technique.*

Seinhorst (1955) described a simple method for separating eelworms from soil, using two Erlenmeyer (conical) flasks each fitted with a funnel. The funnel was held on to the flask by a rubber sleeve, a piece of cycle inner tubing fitted over the outside of the flask neck and the funnel rim (see also Goodey, 1957). This arrangement proved unsatisfactory in use because it was often difficult to attach the funnel to the flask especially when the apparatus was wet. The following method of attachment has been successfully used in the Nematology Department, Rothamsted for the past 3 years and has been found easier to operate than the method described. A plastic funnel is cut down so that its rim just fits inside the rim of the flask. A rubber ring, 3 cm wide and 4 cm diam., made from cycle inner tube, is placed round the funnel rim to leave 0.5 cm inwardly directed overlap (Fig. 1 A). The funnel stem is fitted with a cork or rubber bung of about 4.5 cm diam., fitted with 3 equidistant, round-headed, brass screws held in position by nuts (Fig. 1 A and B). The necks of flasks used are also fitted with rubber rings with 0.5 cm lap over the rim. A length of copper wire, 2 mm diam., with three equally spaced rubber bands is secured around the neck of the flask (Fig. 1 B). The rim of the flasks prevents the wire from slipping off. The funnel is held in position on the flask with one hand while, with the other hand, the rubber bands on the wire ring are stretched and placed over the heads of the screws in the cork round the funnel stem (Fig. 1 B). The overlaps of the rubber rings on the flask and funnel rims accommodate any anomalies in their shape and provide a water-tight union; also the rubber rings help to prevent the rim of the flasks becoming chipped when in use. Care should be taken that the overlap of rubber on the flask is not so wide that soil particles can lodge on it; such lodging can be averted by occasionally tapping the neck of the flask during the extraction operation.

GOODEY, J. B. (1957). *Laboratory methods for work with plant and soil nematodes. Tech. Bull.* **2**, Minist. Agric. London, H.M.S.O. 3rd edition, 47 pp.

SEINHORST, J. W. (1955). Een eenvoudige methode voor het afscheiden van aaltjes uit grond. *Tijdschr. PlZiekt.* **61**, 188-190.

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[1]) Rothamsted Experimental Station, Harpenden Herts., England.

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D. J. HOOPER: *Soil extraction technique.*

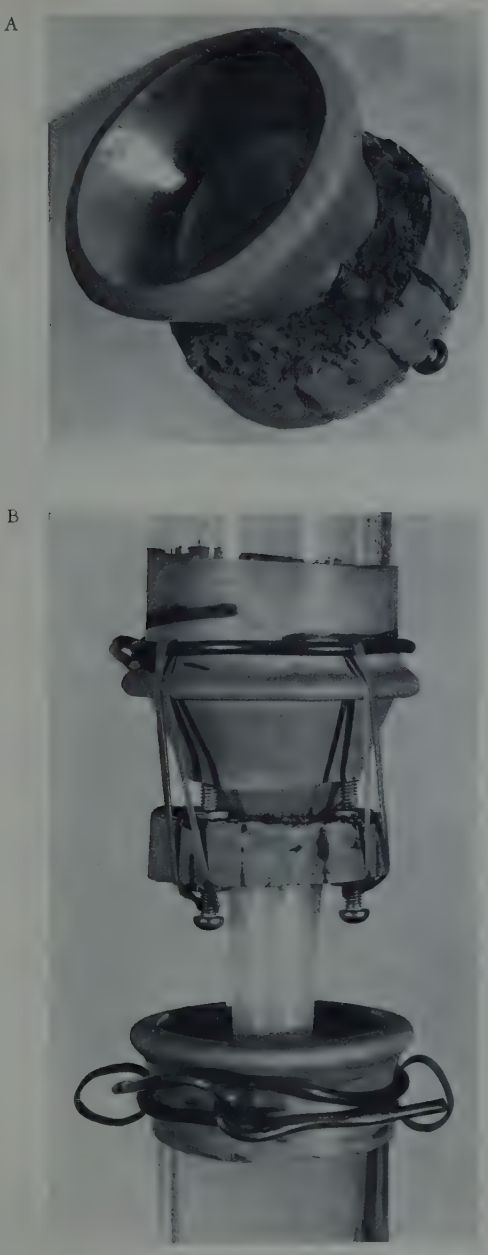

Fig. 1. Adaptation of funnel and flasks for use in the Seinhorst two Erlenmeyer extraction technique: A, plastic funnel fitted with rubber ring and cork. B, funnel attached to flask, the flask below also being adapted for having a funnel fitted to it.

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